OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: November 29, 2017

SUBJECT: **Streptomycin.** Section 3 Registration of Streptomycin on the Citrus Fruit Crop Group 10-10. Summary of Analytical Chemistry and Residue Data.

PC Code: 006310 (sulfate salt) and 006306	**DP Barcode:** D436346
Decision No.: 512067	**Registration No.:** 80990-3 and 80990-4
Petition No.: 5F8427	**Regulatory Action:** Section 3 Registration
Risk Assessment Type: NA	**Case No.:** 0169
TXR No.: NA	**CAS No.:** 3810-74-0 (sulfate salt) and 57-92-1
MRID Nos.: 49785502-49785505	**40 CFR:** §180.245

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1.0 Executive Summary

The Geo Logic Corporation has submitted a petition which requests to establish a tolerance on citrus crop group 10-10 for the antibiotic streptomycin to control bacterial diseases on these crops.

The residue chemistry database is complete for streptomycin and adequate field trial data have been provided to support this proposed new use. Field trials conducted on representative citrus crops are of an adequate number and geographic representation. Data analyses employed validated analytical methods and are supported by adequate storage stability data. The magnitude of the residue data indicate that when following the proposed use pattern, detectable residues of streptomycin are expected in citrus crops at maturity.

A processing study performed on oranges at an exaggerated 3x rate showed that residues concentrate in the processed fractions of dried pulp and peel. Dried pulp is a livestock feedstuff of regulatory

interest associated with citrus crops. For streptomycin, the consumption of feed items potentially bearing residues are expected to be insignificant in relation to its use as a drug in food animals. Nevertheless, citrus dried pulp is an alternative feedstuff only sometimes used in livestock feeding and any resulting residues in livestock commodities are expected to be negligible. Streptomycin thus remains under category 3 of 40 CFR 180.6(a) since there is no reasonable expectation of finite residues in the edible tissues of livestock.

Citrus crops are not grown in rotation and as a result no rotational crop data are required.

2.0 Regulatory Recommendations

The residue chemistry database is adequate for supporting the requested new use of streptomycin on citrus fruit. There are no residue chemistry considerations that preclude establishment of the recommended citrus fruit crop group tolerance.

2.1 Data Deficiencies/Data Needs

There are no residue chemistry deficiencies identified for this petition.

2.2 Tolerance Considerations

2.2.1 Enforcement Analytical Method

To support this requested new use of streptomycin, the registrant has proposed a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS) for tolerance enforcement. This method, "The Analytical Method for the Assay of Streptomycin Residues in Citrus and Citrus Processed Fractions," performs sample extraction using phosphate buffer with pectinase and cellulase. For some citrus processed commodities, 0.1% formic acid is required by the method in the extraction sequence. Following extraction, samples are filtered and brought to volume with phosphate buffer then purified on a solid phase extraction column. Residues are eluted with 1% formic acid in methanol, then evaporated to near dryness, and reconstituted in 0.1% formic acid in water for LC/MS/MS analysis. For the determination of streptomycin, the method monitors the ion transitions of *m/z* 582.2 → 263.2 for quantitation and *m/z* 582.2 → 246.2 for confirmation. The limit of quantitation (LOQ) is 0.01 ppm for all citrus matrices except for dried pulp which is 0.05 ppm all determined as the lowest level of method validation (LLMV). The estimated limit of detection (LOD) is reported to be 0.002 ppm for all citrus matrices except for dried pulp which is reported to be 0.01 ppm.

Conclusions: This method was used for data collection in the supporting field trial studies and adequate validation results were provided in the submission. A successful independent laboratory validation (ILV) study was also provided in the submission. Given these data and the fact that a confirmatory ion transition is also monitored, the Agency concludes that the LC/MS/MS method developed by the registrant is acceptable for streptomycin tolerance enforcement on citrus commodities.

2.2.2 Recommended Tolerances

HED has examined the residue chemistry database for streptomycin. There are no residue chemistry issues that would preclude the establishment of the requested streptomycin citrus fruit tolerances.

Table 2.2.2 summarizes HED's recommendations for the establishing these tolerances under 40 CFR §180.245(a).

Table 2.2.2. Tolerance Summary for Streptomycin, §180.245(a).

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments; *Correct Commodity Definition*
Fruit, citrus, group 10-10	0.50	0.80	
Citrus, dried pulp	3.5	3.0	

In addition, several tolerance modifications are recommended for existing uses of streptomycin for simplification purposes. HED recommends updating the tolerance expression and consolidating permanent tolerances under a single section. Currently, the 40 CFR 180.242 has three subsections under (a), two of these, (2) and (3), specify the type of treatment. This information is not considered necessary. In addition, time-limited tolerances for grapefruit commodities are not necessary. Therefore, we recommend modification and simplification the 40 CFR 180.245 by:

- Removal of the celery, pepper, and tomato tolerances listed under (a)(2), and the potato tolerance listed under (a)(3);
- Establishment of the 0.25 ppm tolerances for tomato, celery, pepper, and potato under the table in (a)(1);
- Removal of the existing time limited tolerances for grapefruit and grapefruit, dried pulp listed under section (b) upon establishment of the permanent tolerances of 0.80 ppm for fruit, citrus, group 10-10 and 3.0 ppm for citrus, dried pulp.

In addition, the 40 CFR §180.245 tolerance expression citations for streptomycin should be updated to be consistent with HED's Interim Guidance on Tolerance Expressions (S. Knizner, 05/27/2009) as follows:

> "(a) *General*. (1) Tolerances are established for residues of the fungicide streptomycin, including its metabolites and degradates, in or on the commodities in the table in this paragraph. Compliance with the tolerance levels specified in this paragraph is to be determined by measuring only streptomycin, *N,N'''*-[(*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-4-[[5-deoxy-2-*O*-[2-deoxy-2-(methylamino)-α-L-glucopyranosyl]-3-*C*-formyl-α-L-lyxofuranosyl]oxy]-2,5,6-trihydroxy-1,3-cyclohexanediyl]bis[guanidine], in or on the commodity."

2.2.3 Revisions to Petitioned-For Tolerances

The citrus commodity tolerances proposed by the petitioner are different from those which are being recommended by HED. This is attributed to the petitioner having input the residue data differently into the calculation procedures for determining the proposed crop group tolerance (including all data for the representative crops into a single calculation). Upon evaluating the data, a tolerance of 0.80 ppm for citrus crop group 10-10 is recommended based on the tolerance determined for grapefruit which gives the highest estimate for the representative citrus fruits. A tolerance of 3.0 ppm for citrus died pulp is recommended based on the highest average field trial (HAFT) residue for grapefruit and the 7x empirical processing factor that was determined. This is in difference to the citrus dried pulp tolerance proposed by the petitioner who used the maximum residue value and not the HAFT for calculation. The petitioner must therefore submit a revised Section F so the proposed tolerances are the same as those recommended by HED.

2.2.4 International Harmonization

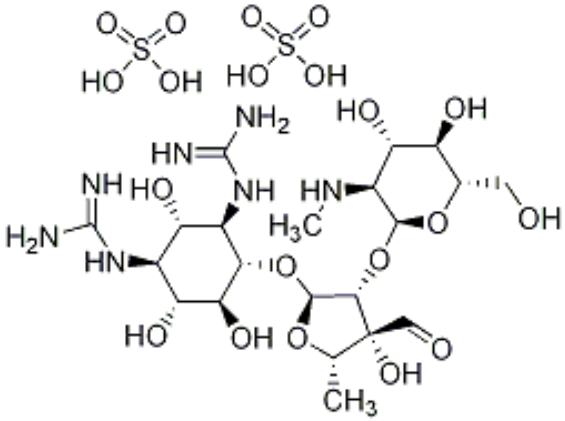
As there are no CODEX or Canadian MRLs established for streptomycin on citrus commodities, no international harmonization issues are expected to arise from establishment of the recommended tolerances (see Appendix A).

2.3 Label Recommendations

None.

3.0 Introduction

3.1 Chemical Identity

Table 3.1. Test Compound Nomenclature.	
Chemical Structure	
Empirical Formula	$C_{42}H_{84}N_{14}O_{36}S_3$
Common Name	Streptomycin Sulfate
Molecular Weight	1457.30356 g/mol
IUPAC Name	1,1-{1-D-(1,3,5/2,4,6)-4-[5-deoxy-2-O-(2-deoxy-2-methylamino-α-L-glucopyranosyl)-3-C-formyl-α-L-lyxofuranosyloxy]-2,5,6-trihydroxycyclohex-1,3-ylene}diguanidine
CAS Name	*N*,*N*′′′-[(*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-4-[[5-deoxy-2-*O*-[2-deoxy-2-(methylamino)-α-L-glucopyranosyl]-3-*C*-formyl-α-L-lyxofuranosyl]oxy]-2,5,6-trihydroxy-1,3-cyclohexanediyl]bis[guanidine]
PC Code	006310
Chemical Abstracts No.	3810-74-0
Registration Review Case No.	0169
Chemical Class	Antibiotic

3.2 Physical/Chemical Characteristics

Physiochemical properties for streptomycin are shown in Table 3.2.

Table 3.2. Physicochemical Properties of Streptomycin

Parameter	Value	Reference
Molecular weight (g/mole)	1467.48 (sulfate salt)	D176743, 6/8/92, C. Swartz
Melting point/range (°C)	168 °C	D176743, 6/8/92, C. Swartz
pH	5.5 (1g sample/ 5 mL water)	D176743, 6/8/92, C. Swartz
Density (g/cm^3)	1.78	D176743, 6/8/92, C. Swartz
Water solubility (at 28°C)	>20 mg/mL	*Merck* 12, 8983
Solvent solubility	Soluble in methanol, ethanol, isopropanol, carbon tetrachloride, and ether	*Merck* 12, 8983
Dissociation constant (pK_a)	7.97	D187344, 5/4/93, C. Swartz
Octanol/water partition coefficient Log(K_{OW})	Waived	

3.3 Pesticide Use Pattern/Directions for Use (860.1200)

A summary of the use directions for the requested new use on citrus crops is provided below in Table 3.3.

Table 3.3. Summary of Directions for Use of the Streptomycin Wettable Powder (WP) Formulations

Application Timing, Type, and Equip.	Formulation [EPA Reg. No.]	Application Rate (lb ai/A)	Max. No. Application per Season	Max. Seasonal Application Rate (lb ai/A)	PHI (days)	RTI (days)
Citrus Crop Group 10-10						
Foliar Spray	FireWall 17 WP [EPA Reg. No. 80990-4] And Agri-Seed 50WP/FireWall 50WP [EPA Reg. No. 80990-3]	0.45	3	1.36	60	21

Conclusions: The use directions are adequate to allow evaluation of the residue data relative to the proposed uses. The proposed maximum application rates, re-treatment intervals, and pre-harvest intervals are supported by the submitted field trial data.

4.0 Metabolite/Degradate Residue Profile

4.1 Nature of the Residue

4.1.1 Summary of Plant Metabolism (860.1300)

Data are not required regarding the nature of the residue of streptomycin in plants because it is already widely used as a human and animal drug (1988 Streptomycin Registration Standard, C. Trichilo).

4.1.2 Summary of Livestock Metabolism (860.1300)

Data are not required regarding the nature of the residue of streptomycin in livestock because it is already widely used as a human and animal drug (1988 Streptomycin Registration Standard, C. Trichilo).

4.1.3 Summary of Confined Rotational Crops (860.1850)

No rotational crop metabolism data are required since citrus is an orchard crop that is not grown in rotation.

4.2 Residues of Concern Summary and Rationale

The 1988 Registration Standard determined that because streptomycin is widely used as a human and animal drug, metabolism data are not needed to support its limited use as a fungicide on crops. HED therefore concluded that the residue of concern for plants and livestock is parent streptomycin only (Table 4.2).

Table 4.2. Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression.

Matrix		Residues included in Risk Assessment	Residues included in Tolerance Expression
Plants	Primary Crop	Streptomycin	Streptomycin
	Rotational Crop	Not Applicable	Not Applicable
Livestock	Ruminant	Streptomycin	Not Applicable[1]
	Poultry		
Drinking Water		Streptomycin	Not Applicable

[1] Tolerances are established in 21 CFR §556.610 for the uncooked edible tissues of chicken, swine, and calves as well as in kidney and other tissues to support the use of streptomycin as an animal drug.

5.0 Residue Profile

5.1 Residue Analytical Methods (860.1340)

5.1.1 Data Collection Methods

49785502.der (citrus RACs)

The proposed enforcement method was used by the registrant for data collection in support of this petition. The method, entitled "The Analytical Method for the Assay of Streptomycin Residues in Citrus and Citrus Processed Fractions" is a high performance LC/MS/MS developed for the determination of streptomycin in citrus commodities. For this method, citrus RACs, juice, and dried pulp are extracted with phosphate buffer along with pectinase and cellulase (pH 4 for fruit & juice, and pH 2 for dried pulp). The resulting sample extract is then filtered and the filtrate is purified on a C8 solid phase extraction (SPE) column. The resulting eluate is adjusted to a pH of 8 and applied to a CBX SPE column for further purification. Residues are then eluted with 1% formic acid in methanol, evaporated to near dryness, and reconstituted in 0.1% formic acid in water for LC/MS/MS analysis. For the preparation of citrus oil, 0.1% formic acid in water is added to the sample and centrifuged. For samples containing detectable residues, a transfer pipette is pushed through the organic layer and an

aliquot of the aqueous layer is collected and diluted with 0.1% formic acid for LC/MS/MS analysis. For citrus oil samples at the LOQ, the supernatant is evaporated to near dryness and diluted with 0.1% formic acid for LC/MS/MS analysis. The method determines streptomycin by monitoring the ion transition of *m/z* 582.3 → 263.1 for quantitation. The LOQ of the method is 0.01 ppm and the LOD is 0.002 ppm for all citrus matrices except dried pulp which has an LOQ of 0.05 ppm and an LOD of 0.01 ppm.

Method performance was evaluated using untreated samples of citrus fruit, juice, peel, dried pulp, and oil fortified with streptomycin for method validation and concurrent recovery testing. For these analyses, the untreated samples were fortified at 0.01 and 0.1 ppm for citrus fruit, juice, and peel, 0.05, 0.5, and 1.0 ppm for dried pulp, and 0.01, 0.1, and 1.0 ppm for oil. These fortification levels appropriately bracketed the measured residues. The detector responses were linear with a reported correlation coefficient of $r^2 \geq 0.9946$ within the calibration range of 1.0-100 ng/mL. Representative chromatograms of control samples, fortified samples, and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Apparent residues in controls were below the LOQ for all citrus samples. The method was demonstrated to be adequate with all method validation and concurrent recoveries all being reported within the acceptable range of 70-120%.

5.1.2 Multi-Residue Methods (860.1360)

Testing of streptomycin through multi-residue methods (MRM) in the Pesticide Analytical Manual (PAM), Vol. I is not practical since streptomycin is not sufficiently volatile for GLC applications (1988 Streptomycin Registration Standard, C. Trichilo).

5.1.3 Tolerance Enforcement Methods

49785503.der (ILV Study)
49785504.der (ILV Study Report Amendment)
49785505.der (Tolerance Enforcement Method)

"The Analytical Method for the Assay of Streptomycin Residues in Citrus and Citrus Processed Fractions" is concluded to be an acceptable method for the tolerance enforcement of citrus commodities. A complete description of the method was included in the submission. The method as written was adequately validated by the registrant using fortified samples of citrus whole fruit, citrus whole fruit no peel, citrus peel, orange juice, orange dried pulp, and orange oil. For these analyses, the samples were fortified at 0.01 and 0.1 ppm for citrus whole fruit, citrus whole fruit no peel, citrus peel, and orange juice, at 0.05 and 0.5 ppm for orange dried pulp, and at 0.01, 0.1, and 1.0 ppm for orange oil. Recoveries were within the acceptable range of 70-120% with a relative standard deviation of 0.96-14.41 for these analyses. A successful Independent Laboratory Validation (ILV) was also conducted by Ricerca Biosciences, LLC using samples of untreated whole oranges fortified with streptomycin at 0.01 and 0.50 ppm. The ILV laboratory reported that the validation passed but also offered modifications for improving recovery and reducing background interference. No radiovalidation data were included in the submission. The method does monitor two ion transitions at *m/z* 582.2 → 263.2 for quantitation, and *m/z* 582.2 → 246.2 for confirmation of streptomycin. Therefore, no additional confirmatory procedures are needed.

5.1.4 Submittal of Analytical Reference Standards (860.1650)

Analytical standards for streptomycin are not available in the EPA National Pesticide Standards Repository (personal communication with Gregory Verdin, BEAD, 08/30/2016). A reference standard for streptomycin must be provided to the Repository, and then replenished as requested by the Repository. The reference standard should be sent to the Analytical Chemistry Lab, which is located at Fort Meade, to the attention of Theresa Cole at the following address:

USEPA
National Pesticide Standards Repository/Analytical Chemistry Branch/OPP
701 Mapes Road
Fort George G. Meade, MD 20755-5350

The full 9 digit zip code is mandatory or the mail will be returned.

5.2 Storage Stability (860.1380)

The storage interval of the citrus crop field trial samples ranged from 75 to 184 days. The storage interval of the samples for the citrus processing study ranged from 131 to 213 days. Storage stability data were generated concurrently in support of these studies. The concurrent studies that were conducted demonstrated stability for an interval of up to 50 days in orange whole fruit and 49 days in orange whole fruit without peel and in peel. No 0-day data were provided. There are additional storage stability data available which demonstrate that residues of streptomycin are stable in frozen grapefruit for up to 327 days in the RAC, 481 days in juice, and 720 days in dried pulp (MRID No. 49229401). In regard to oil, recoveries were reported only to be 49% at 474 days and 36% at 502 days for this study. There are no 0-day data provided and only the results determined for the final storage intervals are reported. A summary of the storage durations and conditions of the samples from the crop field trials and processing studies provided to support this action presented below in Table 5.2 for review.

Table 5.2. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability
Citrus Crop Field Trial Samples			
Citrus, whole fruit	<-10	75-184 days (2.5-6.1 months)	Residues of streptomycin are stable for up to 50 days (1.7 months) in/on whole fruit, and 49 days (1.6 months) in/on whole fruit without peel and in/on peel, respectively.[2] In addition, acceptable concurrent storage stability data are available indicating that residues of streptomycin in grapefruit are stable during frozen storage for up to 327 days (10.9 months) in the RAC.[3]
Citrus, whole fruit without peel		93-107 days (3.1-3.5 months)	
Citrus, peel		100-113 days (3.3-3.7 months)	

Table 5.2. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability
Citrus Processing Study Samples			
Orange (RAC)	-21 to -16 (processing facility) <-10 (laboratory)	213 days (7.0 months)	Residues of streptomycin are stable for up to 50 days (1.7 months) in/on whole fruit, and 49 days (1.6 months) in/on whole fruit without peel and in/on peel, respectively.[2] In addition, acceptable concurrent storage stability data are available indicating that residues of streptomycin in grapefruit are stable during frozen storage for up to 327 days (10.9 months) in the RAC, 481 days (16 months) in juice, and 720 days (24 months) in dried pulp. For this grapefruit storage stability study, recoveries for oil were reported to be 49% at 474 days (15.8 months) and 36% at 502 days (16.7 months).[3]
Juice		131 days (4.3 months)	
Dried pulp		213 days (7.0 months)	
Oil		147 days (4.8 months)	

[1] Interval from harvest to extraction or processing. Samples were analyzed within 0-1 days of extraction.
[2] Based on concurrent storage stability study. See MRID No. 49785502.
[3] Refer to MRID No. 49229401.

Conclusions. Adequate storage stability data are available to support the sample storage intervals incurred in the magnitude of the residue and processing studies submitted for this petition. Although the prior storage stability data acquired for grapefruit indicates there may be some instability in oil, the interval reported for this data is 327 days (10.9 months) longer in duration then what is needed to support the oil samples held in the current study. Any residue decline in the processed oil samples realized at the 147 day (4.8 month) storage interval would not be significant in consideration of these data.

5.3 Residue Data

5.3.1 Crop Field Trials (860.1500)

49785502.der (citrus RACs)

AgroSource, Inc. has submitted field trial data for determining the magnitude of residue of streptomycin in/on citrus RACs. For this study, a total of twenty-four (24) citrus fruit field trials were conducted in the United States during the 2014 growing season. It consists of five (5) field trials for lemon in North American Free Trade Agreement (NAFTA) Growing Zones 3 (FL; 1 trial) and 10 (CA; 4 trials), six (6) field trials for grapefruit in NAFTA Growing Zones 3 (FL; 3 trials), 6 (TX, 1 trial), and 10 (CA, 2 trials), and thirteen (13) orange trials in NAFTA Growing Zones 3 (FL; 9 trials), 6 (TX, 1 trial), and 10 (CA, 3 trials).

Each trial site consisted of one untreated and one treated test plot. At each trial location, the treated test plots received three foliar directed airblast applications of a 50% wettable powder (WP) formulation of streptomycin (FireWall^{TM} 50 WP) at 0.447-0.462 lb ai/A/application for a total seasonal rate of 1.347-1.375 lb ai/A. Applications were made at re-treatment intervals (RTIs) of 20-21 days using ground equipment in spray volumes of ~91-110 gal/A. No adjuvants were used. Samples of lemon, grapefruit, and orange were harvested at maturity at a pre-harvest interval (PHI) of 60-62 days. At one trial each for grapefruit and orange, samples were harvested at additional PHIs of 40, 50, 70, and 80 days to assess residue decline.

Following treatment, the citrus RAC samples were analyzed for residues of streptomycin using a high performance LC/MS/MS method. Residues of streptomycin ranged from <0.01-0.089 ppm in/on

lemon, <0.01-0.478 ppm in/on grapefruit, and <0.01-0.152 ppm in/on orange. In comparison, residues were <0.01-0.478 ppm in/on whole citrus fruit, <0.01-0.017 ppm in/on whole fruit without peel, and <0.01-1.12 ppm in/on peel. In the residue decline trials, residues of streptomycin decreased to LOQ (<0.01 ppm) in/on grapefruit and decreased with increasing PHI in/on orange. A summary of the field trial results is shown in Table 5.3.1, below.

Table 5.3.1. Summary of Residues from Citrus Fruit Trials with Streptomycin.

Crop Matrix	Total Application Rate (lb ai/A)	PHI (days)	n[1]	Residues (ppm)						
				Min.[2]	Max.[2]	LAFT[3]	HAFT[3]	Median[3]	Mean[3]	SD[3]
Lemon	1.351-1.363	60-62	5	<0.01	0.089	<0.01	0.069	0.021	0.033	0.027
Grapefruit	1.347-1.375	60-62	6	<0.01	0.478	<0.01	0.414	0.029	0.089	0.159
Orange	1.352-1.372	60-62	13	<0.01	0.152	<0.01	0.144	0.061	0.064	0.041
Citrus, whole fruit	1.347-1.375	60-62	24	<0.01	0.478	<0.01	0.414	0.037	0.064	0.083
Citrus, whole fruit without peel			22	<0.01	0.017	<0.01	0.016	0.010	0.011	0.0015
Citrus, peel			22	<0.01	1.12	<0.01	1.08	0.091	0.186	0.266

[1] n = number of field trials.
[2] Values based on total number of samples.
[3] Values based on per-trial averages. LAFT = lowest average field trial, HAFT = highest average field trial, SD = standard deviation. For computation of the LAFT, HAFT, median, mean, and standard deviation, values < LOQ are assumed to be at the LOQ (0.01 ppm).

Conclusions. The submitted citrus fruit field trial data are acceptable, as an acceptable method was used for residue quantitation, and adequate storage stability data are available to support sample storage durations and conditions. Further, the number of trials and their distribution are appropriate for the establishment of a citrus fruit crop group tolerance.

5.3.2 Field Rotational Crops (860.1900)

Field rotational crop metabolism data are not required since citrus is an orchard crop that is not grown in rotation.

5.3.3 Processed Food and Feed (860.1520)

49785502.der (Processing Study)

A processing study performed on oranges at an exaggerated 3x rate showed that residues concentrate in the processed fractions of dried pulp and peel. In processed citrus commodities, average residues found in the study were below the LOQ (<0.01 ppm) in juice and oil, 0.681 ppm in dried pulp, and 0.236 ppm in peel. A comparison of the residues in whole fruit (RAC) with those in the processed orange fraction (juice, dried pulp, oil, and peel) indicated that residues of streptomycin concentrate in dried pulp (processing factor of 6.9x) and peel (2.4x) but do not concentrate in juice or oil (<0.1x). A summary of the processing study results is shown in Table 5.3.3, below.

Table 5.3.3. Residue Data from Orange Processing Study with Streptomycin.

Commodity	Residues[1] (ppm) [Average]	Processing Factor[2]	Median Processing Factor[3]
Orange (RAC)	0.101, 0.095 [0.098]	--	--
Juice	(0.00406), (0.00573) [<0.01]	<0.1x	Not applicable
Dried pulp	0.573, 0.788 [0.681]	6.9x	
Oil[4]	(0.00264), (0.00831) [<0.01]	<0.1x	
Peel	0.214[5], 0.258[6] [0.236]	2.4x	

[1] The LOQ was 0.01 ppm, and the LOD was 0.002 ppm for all matrices, except orange dried pulp. The LOQ was 0.05 ppm, and the LOD was 0.01 ppm for orange dried pulp. Residues between the LOD and LOQ are reported in parentheses. Per-trial averages were calculated by the study reviewer using the LOQ for values reported as <LOQ.

[2] Processing Factor = [Measured residue for analyte in the processed fraction] / [Measured residue for analyte in the RAC].
[3] Median processing factor for both plots and all sites.
[4] The available storage stability data indicate that residues of streptomycin were not stable in oil (recoveries were 49% at 474 days and 36% at 502 days); however, residues were not corrected for loss on storage because residues were below the LOQ.
[5] Mean of duplicate dilution analyses.
[6] Result of one dilution only.

Conclusions: Processing studies performed on oranges are acceptable in demonstrating that residues of streptomycin concentrate in dried pulp (processing factor of 6.9x) and peel (2.4x).

5.3.4 Meat, Milk, Poultry and Eggs (860.1480)

Dried pulp is a livestock feedstuff of regulatory interest associated with citrus crops. It is an alternative feedstuff only sometimes used in livestock feeding. Although citrus dried pulp may contain residues of streptomycin, concentrations are expected to be negligible in relation to its use as a drug in food animals. Streptomycin therefore remains under category 3 of 40 CFR 180.6(a) since there is no reasonable expectation of finite residues in the edible tissues of livestock.

5.4 Food Residue Profile

Adequate residue chemistry data have been provided for streptomycin. Field trials are of adequate number and geographic representation. Data analyses employed validated analytical methods and are supported by adequate storage stability data. The LOQ for all citrus commodities proposed in the current action is 0.01 ppm except for dried pulp which has an LOQ of 0.05 ppm. The magnitude of the residue data indicate that when following the proposed use pattern, detectable residues of streptomycin are expected in citrus RACs. Empirical processing data indicate that residues of streptomycin concentrate in dried pulp (processing factor of 6.9x) and peel (2.4x) following treatment at an exaggerated rate of 3x. In consideration of these data a separate tolerance is needed for citrus dried pulp. Because there is no reasonable expectation of finite residues in livestock, streptomycin therefore remains under category 3 of 40 CFR 180.6(a). Rotational crop data are not required for streptomycin because citrus is an orchard crop that is not grown in rotation. Recommended tolerances are based on the newly acquired field trial data analyzed for the representative citrus RACs. No additional residue chemistry data are required.

6.0 Tolerance Derivation

The recommended tolerance for streptomycin in or on citrus RACs was derived by use of Organization for Economic Cooperation and Development (OECD) Maximum Residue Limit (MRL) calculation procedures and consideration of international MRLs (see Appendices A and B). A tolerance of 0.80 ppm is recommended for citrus fruit crop group 10-10 based on the MRL calculated for grapefruit which gave the highest estimate in comparison to the other representative citrus crops. For citrus dried pulp, a tolerance of 3.0 ppm is recommended based on the product of the HAFT and the empirical processing factor that was determined for this commodity. No international harmonization issues are expected to arise from establishment of the recommended tolerances

References

Trichilo, C. (1988). Streptomycin Registration Standard.

Homa, K. (2012). MRID No. 49229401. Streptomycin: Magnitude of the Residue on Grapefruit. IR-4 Study Number: 10043.08-FLR09. Unpublished study prepared by IR-4. 285 p.

List of Appendices

A. International Residue Limits Table.
B. Tolerance Calculations.

Appendix A. International Residue Limits Table

(006310; 09/07/2016)

Summary of US and International Tolerances and Maximum Residue Limits				
Residue Definition:				
US		Canada	Mexico	Codex
§ 180.245 (a) General. Streptomycin; *N*,*N*'''-[(*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-4-[[5-deoxy-2-*O*-[2-deoxy-2-(methylamino)-α-L-glucopyranosyl]-3-*C*-formyl-α-L-lyxofuranosyl]oxy]-2,5,6-trihydroxy-1,3-cyclohexanediyl]bis[guanidine]		None		None
Commodity	*Tolerance (ppm) /Maximum Residue Limit (mg/kg)*			
	US	Canada	Mexico	Codex
Fruit, citrus, group 10-10	0.4			
Citrus, dried pulp	3.5			
Completed: M. Negussie; 09/08/2016				

Appendix B. Tolerance Calculations

Streptomycin
Grapefruit, Citrus Crop Group 10-10
U.S.
PHI @ 60-days

Total number of data (n)	6
Percentage of censored data	17%
Number of non-censored data	5
Lowest residue	0.010
Highest residue	0.414
Median residue	0.030
Mean	0.090
Standard deviation (SD)	0.159
Correction factor for censoring (CF)	0.889
Proposed MRL estimate	
- Highest residue	0.414
- Mean + 4 SD	0.726
- CF x 3 Mean	0.239
Unrounded MRL	0.726
Rounded MRL	**0.8**

High uncertainty of MRL estimate.
[Small dataset]

Residues (mg/kg)	n
< 0.01	1
0.02	1
0.027	1
0.032	1
0.035	1
0.414	1

Streptomycin
Orange, Citrus Crop Group 10-10
U.S.
PHI @ 60-days

Total number of data (n)	13
Percentage of censored data	8%
Number of non-censored data	12
Lowest residue	0.010
Highest residue	0.144
Median residue	0.061
Mean	0.064
Standard deviation (SD)	0.041
Correction factor for censoring (CF)	0.949

Proposed MRL estimate

- Highest residue	0.144
- Mean + 4 SD	0.227
- CF x 3 Mean	0.182
Unrounded MRL	0.227
Rounded MRL	**0.3**

Residues (mg/kg)	n
< 0.01	1
0.02	1
0.022	1
0.032	1
0.038	1
0.05	1
0.061	1
0.068	1
0.076	1
0.089	1
0.102	1
0.118	1
0.144	1

Streptomycin
Lemon, Citrus Crop Group 10-10
U.S.
PHI @ 60-days

Total number of data (n)	5
Percentage of censored data	40%
Number of non-censored data	3
Lowest residue	0.010
Highest residue	0.069
Median residue	0.021
Mean	0.033
Standard deviation (SD)	0.027
Correction factor for censoring (CF)	0.733

Proposed MRL estimate

- Highest residue	0.069
- Mean + 4 SD	0.142
- CF x 3 Mean	0.073
Unrounded MRL	0.142
Rounded MRL	**0.15**

High uncertainty of MRL estimate.
[Small dataset]

Residues (mg/kg)	n
< 0.01	2
0.021	1
0.055	1
0.069	1

Volume 3

Annex B

Streptomycin

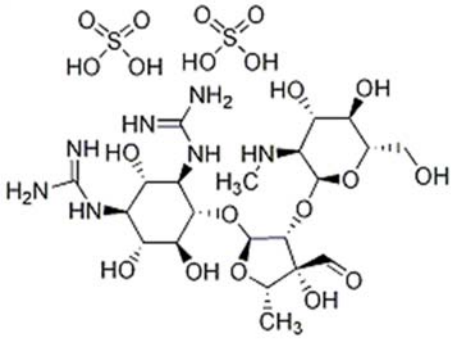
B.5 METHODS OF ANALYSIS

B.5.2 ANALYTICAL METHODS FOR THE DETERMINATION OF RESIDUES (ALL COMPONENTS INCLUDED IN THE RESIDUE DEFINITION PROPOSED)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 6/22/16). The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

Table B.5.2-1. Overview of the Analytical Methods for the Determination of Streptomycin Residues.

Data Requirement	Matrix	Analytes	Method Type	Limit of Quantitation (ppm)	Reference
Enforcement Method- Plant Commodities	Citrus and processed fractions	Streptomycin	LC/MS/MS Analytical Method for Streptomycin Residues in Citrus and Citrus Processed Fractions	0.01, all citrus commodities except dried pulp 0.05, dried pulp	Study Nos. 033236-1 and 033040 MRIDs 49785502 and 49785505 [DP# 431343] PMRA# not applicable
Data-Gathering Method- Plant Commodities	Citrus and processed fractions	Streptomycin	LC/MS/MS Analytical Method for Streptomycin Residues in Citrus and Citrus Processed Fractions	0.01, all citrus commodities except dried pulp 0.05, dried pulp	Study No. 033040 MRID 49785502 [DP# 431343] PMRA# not applicable
ILV of Enforcement Method- Plant Commodities	Orange	Streptomycin	LC/MS/MS (Analytical Method Report No. 033041-1; Analytical Method for Streptomycin Residues in Citrus)	0.01	Study No. 033039 MRIDs 49785503 and 49785504 [DP# 431343] PMRA# not applicable
Radiovalidation of Methods - Plant Commodities				N/A	Not Available
Enforcement Method- Livestock Commodities					Not Available
Data-Gathering Method- Livestock Commodities					Not Available
ILV of Enforcement Method- Livestock Commodities					Not Available
Radiovalidation of Methods - Livestock Commodities					Not Available
Multiresidue Method Testing			FDA-Pesticide Analytical Methods Multi-Residue testing program		Not Available

Table B.5.2-2. Chemical Structures of Analytes Addressed by Methods for the Analysis of Streptomycin.		
Method ID	Chemical Name	Chemical Structure
Analytical Method for Streptomycin Residues in Citrus and Citrus Processed Fractions	**Common Name:** Streptomycin **Chemical Name:** 1,1-{1-L-(1,3,5/2,4,6)-4-[5-deoxy-2-O-(2-deoxy-2-methylamino-α-L-glucopyranosyl)-3-C-formyl-α-L-lyxofuranosyloxy]-2,5,6-trihydroxycyclohex-1,3-ylene}diguanidine	

B.5.2.1 Analytical Methods for Plant Matrices (Annex IIA 4.3, Annex IIIA 5.3)

B.5.2.1.1 Post-Registration Method (Enforcement)

Document ID: MRID Nos. 49785502, 49785503, 49785504, and 49785505
PMRA No. Not Applicable

Report: 49785502 Gibson, T. (2015) Magnitude of the Residues of Streptomycin in/on Citrus and Processed Fractions of Citrus Following Applications of FireWall™ 50 WP. Study ID Nos.: PSM-14-03-02 and 033040. Unpublished study prepared by AgroSource, Inc. 349 p.

49785503 Panter, L. (2015) Independent Laboratory Validation (ILV) Study of an Analytical Method for Streptomycin Residues in Citrus. Study ID No.: 033039. Unpublished study prepared by Geo Logic Corporation. 85 p.

49785504 Panter, L. (2015) Report Amendment Independent Laboratory Validation (ILV) Study of an Analytical Method for Streptomycin Residues in Citrus. Study ID No.: 033039-1-1. Unpublished study prepared by Geo Logic Corporation. 85 p.

49785505 Cassidy, P. (2015) Streptomycin Citrus Enforcement Method. Study ID No.: 033236-1. Unpublished study prepared by Geo Logic, Inc. 28 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1340 Residue Analytical Method (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 3 – Residue Analytical Method
EU SANCO 825/00/rev. 7 (17/3/04)
OECD Guidance Document on Pesticide Residue Analytical Methods

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#431343.

Evaluator: Peter Savoia HED/RAB V

EXECUTIVE SUMMARY

AgroSource, Inc. has submitted an analytical method description and validation data (MRIDs 49785502 and 49785505) for a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), for the determination of residues of streptomycin in/on citrus and processed fractions. In addition, Geo Logic Corporation has submitted the results of an independent laboratory validation (ILV; MRIDs 49785503 and 49785504) of the method conducted by Ricerca Biosciences, LLC (Concord, OH).

Briefly, samples are extracted with phosphate buffer (pH 4) and pectinase and cellulase, filtered, and brought to volume with phosphate buffer (pH 4). An aliquot of the extract is purified on a C8 SPE column. The resulting eluate is adjusted to pH ~8 and applied to a cation exchange (CBX) SPE column for further purification. Residues are eluted with 1% formic acid in methanol. The eluate is evaporated to near dryness and reconstituted in 0.1% formic acid in water for LC/MS/MS analysis. The method monitors the following ion transition for determination of streptomycin: *m/z* 582.2 → 263.2 (quantitation) and *m/z* 582.2 → 246.2 (confirmation). The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) is 0.01 ppm for all citrus matrices except orange dried pulp which had an LOQ of 0.05 ppm. The estimated limit of detection (LOD) was 0.002 ppm for all citrus matrices except orange dried pulp which had an LOD of 0.01 ppm. The method does not provide for conversion of residues of streptomycin sulfate to streptomycin. The concentration of the stock solution should be converted from streptomycin sulfate to streptomycin by multiplying by the molecular weight conversion factor of 0.7977 or 79%.

The method was adequately validated for the quantitation ion transitions using samples of citrus whole fruit, citrus whole fruit no peel, citrus peel, orange juice, orange dried pulp, and orange oil. Samples were fortified with streptomycin at 0.01 and 0.1 ppm for citrus whole fruit, citrus whole fruit no peel, citrus peel, and orange juice, at 0.05 and 0.5 ppm for orange dried pulp, and at 0.01, 0.1, and 1.0 ppm for orange oil. Recoveries were generally within the acceptable range of 70-120%. Mean recoveries (and relative standard deviations) for streptomycin were: 74-102% (4.01-10.93%) for citrus, whole fruit; 98-113% (0.96-8.76%) for citrus, whole fruit no peel; 71-82% (2.58-6.78%) for citrus peel; 95.5-107.4% (4.01-5.03%) for orange juice; 69.8-84.9% (7.49-7.58%) for orange, dried pulp; and 92.7-98.1% (6.37-14.41%) for orange oil. Repeatability data were generated from three samples fortified at the LOQ and 10x LOQ for each matrix and also at 100x LOQ for orange oil.

The ILV of Analytical Method Report No. 033041-1 (Analytical Method for Streptomycin Residues in Citrus) was conducted by Ricerca Biosciences, LLC (Concord, OH) using samples of untreated whole oranges fortified with streptomycin at 0.01 and 0.5 ppm (LOQ and 50x the LOQ/proposed tolerance, respectively).

There were several communications between the Study Director and the Sponsor Representative during the investigation, which began prior to the start of the ILV and continued through the entire study. Based on initial consultation with the Sponsor Representative concerning some recoveries at the LOQ that were below the acceptable range, the Study Director suspected that the low recovery may be due to some matrix suppression and injected at a lower injection volume to alleviate that concern. The results indicated that recoveries improved at the lower injection volume. In addition, the Study Director indicated there is some elevated background

interference with the confirmatory ion in the control sample which may contribute to a peak area at the LOQ level leading to a higher recovery in comparison to quantitation ion. After discussing if using dilution would be helpful to reduce the background, the Study Director agreed that dilution would help to reduce the background, but the observed variation (~10%) between the quantitation and confirmatory ions would remain acceptable considering the range of acceptability (70-120%).

The ILV was successful on the first attempt; recoveries were generally within the acceptable range of 70-120% at both fortification levels for both the quantitation and confirmation ion transitions. Apparent residues of streptomycin were nondetectable (<0.002 ppm) in/on two unfortified samples of orange for both the quantitation and confirmation ion transitions.

The ILV laboratory reported that a total of 16 worker hours were required to prepare and analyze the set of samples by LC/MS/MS (including standard preparation and analysis). Two calendar day were required for one set of samples.

No radiovalidation data were included in the submission. Analytical methods should be radiovalidated to determine whether total toxic residues (TTR) are extracted from weathered matrices.

I. Principle of the Method: Analytical Method for the Assay of Streptomycin in Citrus

Table B.5.2.1.1-1. Summary Parameters for the Post-Registration Analytical Method for the Analysis of Streptomycin Residues in Citrus.

Method ID	No Method ID number available Streptomycin Citrus Enforcement Method Analytical Method for the Assay of Streptomycin Residues in Citrus and Citrus Processed Fractions
Analyte(s)	Streptomycin
Extraction solvent and technique	Briefly, samples are extracted with phosphate buffer (pH 4) and pectinase and cellulase, filtered, and brought to volume with phosphate buffer (pH 4).
Clean-up strategies	An aliquot of the extract is purified on a C8 SPE column. The resulting eluate is adjusted to pH ~8 and applied to a cation exchange (CBX) SPE column for further purification. Residues are eluted with 1% formic acid in methanol. The eluate is evaporated to near dryness and reconstituted in 0.1% formic acid in water for LC/MS/MS analysis.
Instrument and Detector	LC/MS//MS using a Phenomenex Hyperclone C18 column; MS/MS detection using multiple reaction monitoring (MRM) with Turbo Spray ionization in the positive mode. The method monitors the following ion transition for determination of streptomycin: *m/z* 582.2 → 263.2 (quantitation) and *m/z* 582.2 → 246.2 (confirmation).
Standardization method	External standardization using calibration standards to generate a seven-point calibration curve (quadratic regression, 1/x weighted) over the range of 1-100 ng/mL.
Stability of std solutions	Not addressed. Store the stock solution at approximately -20 °C when not in use.
Retention times	~5.4 minutes

The method does not provide for conversion of residues of streptomycin sulfate to streptomycin. The concentration of the stock solution should be converted from streptomycin sulfate to streptomycin by multiplying by the molecular weight conversion factor of 0.7977 or 79%.

II. Specificity

The LC/MS/MS method is highly selective for the quantitation of streptomycin. Analysis of control samples resulted in no significant signals at the expected retention times of the analyte. The following ion transitions are monitored:

Streptomycin	*m/z* 582.2→263.2 (quantitation)
	m/z 582.2→246.2 (confirmation)

Because the method monitors multiple ion transitions for determination of streptomycin, no additional confirmatory procedures are needed.

Confirmation is accomplished by comparison of the retention times and the selective reaction monitoring (SRM) using up to two transition ions MS/MS spectra with those of fortified samples and external standards.

III. Linearity

The linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by quadratic regression analysis. For the least-square equation, which describes the detector response as a function of the standard concentration, a representative calibration curve resulting from the injection of a minimum of seven standards over the concentration range of 1-100 ng/mL demonstrated linearity with coefficients of determination (r^2) of at least 0.9992 (quantitation) and 0.9978 (confirmation).

IV. Accuracy (Recovery) and Precision (Repeatability)

Radiovalidation. No radiovalidation data were included in the submission. These data are required to demonstrate the ability of the method to extract incurred residues.

Method validation. The method was adequately validated for the quantitation ion transitions using samples of citrus whole fruit, citrus whole fruit no peel, citrus peel, orange juice, orange dried pulp, and orange oil. Samples were fortified with streptomycin at 0.01 and 0.1 ppm for citrus whole fruit, citrus whole fruit no peel, citrus peel, and orange juice, at 0.05 and 0.5 ppm for orange dried pulp, and at 0.01, 0.1, and 1.0 ppm for orange oil. Recoveries were generally within the acceptable range of 70-120%. Mean recoveries (and relative standard deviations) for streptomycin were: 74-102% (4.01-10.93%) for citrus, whole fruit; 98-113% (0.96-8.76%) for citrus, whole fruit no peel; 71-82% (2.58-6.78%) for citrus peel; 95.5-107.4% (4.01-5.03%) for orange juice; 69.8-84.9% (7.49-7.58%) for orange, dried pulp; and 92.7-98.1% (6.37-14.41%) for orange oil. Repeatability data were generated from three samples fortified at the LOQ and 10x LOQ for each matrix and also at 100x LOQ for orange oil. Recovery and repeatability data are presented in Table B.5.2.1.1-2, below.

Table B.5.2.1.1-2. Accuracy and Precision Data for the Validation of Analytical Method for the Assay of Streptomycin in Citrus.[1]

Analyte	Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)[2]
			Individual	Mean	Range	
Streptomycin *m/z* 582.2→263.2 (quantitation)	Citrus, whole fruit	0.01	64, 76, 84	74	64-84	10.93
		0.1	98, 108, 101	102	98-108	4.01
	Citrus, whole fruit no peel	0.01	114, 114, 112	113	112-114	0.96
		0.1	104, 86, 105	98	86-105	8.76
	Citrus peel	0.01	74, 71, 70	71	70-74	2.58
		0.1	76, 89, 80	82	76-89	6.78
	Orange juice	0.01	104, 103, 115	107	103-115	5.03
		0.1	100, 96, 91	95.5	91-100	4.01
	Orange dried pulp	0.05	62.6, 71.7, 75.1	69.8	62.6-75.1	7.58
		0.5	75.9, 88.6, 90.1	84.9	75.9-90.1	7.49
	Orange oil	0.01	90.8, 87.9, 101.7	93.4	87.9-101.7	6.37
		0.1	101.5, 93.4, 83.1	92.7	83.1-101.5	8.11
		1.0	104.5, 111.4, 78.5	98.1	78.5-111.4	14.41

[1] Method validation data were also reported in the crop field trial and processing DERs (refer to 49785502.de1 and 49785502.de2, respectively).
[2] As reported by the petitioner.

Independent laboratory validation (MRIDs 49785503 and 49785504). The ILV of Analytical Method Report No. 033041-1 (Analytical Method for Streptomycin Residues in Citrus) was conducted by Ricerca Biosciences, LLC (Concord, OH) using samples of untreated whole oranges fortified with streptomycin at 0.01 and 0.5 ppm (LOQ and 50x the LOQ/proposed tolerance, respectively) and analyzed according to the method procedures described in Table B.5.2.1.1-1.

There were several communications between the Study Director and Sponsor Representative pertaining to the ILV. These are listed below:

- Prior to starting the ILV, the Study Director asked if the 1000 ng/mL sample could be removed from the calibration curve because they were encountering some carry over from that sample and suspected the it was only an intermediate dilution and not used for the calibration curve. The Sponsor Representative approved the suggested approach.
- The Study Director provided the raw data and Excel spreadsheet for the first trial and the Study Representative noted that some of the recoveries at the LOQ were below the acceptable range and inquired if those values would be acceptable. The Sponsor Representative replied with an excerpt from EPA guidelines OCSPP 860.1340 “The Agency will consider the variability in recovery values when determining the acceptability of methods with recoveries outside the 70-120 percent range. For example, a method with average recovery of 65 percent and a low CV (e.g., 5 percent) may be viewed more favorably than a method showing 95 percent average recovery and a CV greater than 20 percent." The Study Director indicated that the low recovery may be due to some matrix suppression and would inject at a lower injection volume to alleviate that concern.
- The Study Director provided the results (raw data and Excel spreadsheet) at the lower injection volume and the recoveries were improved. The Study Director indicated there is some elevated background interference with the confirmatory ion in the control sample which may contribute to a peak area at the LOQ level leading to a higher recovery in comparison to quantitation ion. The Sponsor Representative asked if dilution would help

to reduce the background. The Study Director agreed that dilution would help to reduce the background, but the observed variation (~10%) between the quantitation and confirmatory ions would remain acceptable considering the range of acceptability (70-120%).

The results of the ILV are presented in Table B.5.2.1.1-3. The ILV was successful on the first attempt; recoveries were generally within the acceptable range of 70-120% at both fortification levels for both the quantitation and confirmation ion transitions. Apparent residues of streptomycin were nondetectable (<0.002 ppm) in/on two unfortified samples of orange for both the quantitation and confirmation ion transitions.

The ILV laboratory reported that a total of 16 worker hours were required to prepare and analyze the set of samples by LC/MS/MS (including standard preparation and analysis). Two calendar day were required for one set of samples.

Table B.5.2.1.1-3. Accuracy and Precision Data for the Independent Laboratory Validation of RAM Method Report No. 033041-1 (Analytical Method for Streptomycin Residues in Citrus) for Orange.

Analyte	Matrix	Fortification Level (ppm)	Independent Laboratory Validation Recovery (%)			RSD (%)
			Individual	Mean	Range	
Streptomycin *m/z* 582.2→263.2 (quantitation)	Orange, whole	0.01	69.9, 74.4, 75.5, 75.6, 68.3	72.7	68.3-75.6	4.7
		0.5	78.3, 81.8, 71.3, 77.9, 78.1	77.5	71.3-81.8	4.9
		Overall		75.1	68.3-81.8	5.6
Streptomycin *m/z* 582.2→246.2 (confirmation)	Orange, whole	0.01	85.4, 85.7, 94.6, 95.9, 81.4	88.6	81.4-95.9	7.1
		0.5	75.6, 83.0, 74.2, 77.0, 81.4	78.3	74.2-83.0	4.8
		Overall		83.4	74.2-95.9	8.8

V. Limit of Quantitation

Table B.5.2.1.1-4. Summary of Detection and Quantitation Limits for Analytical Method for the Assay of Streptomycin in Citrus.

Analyte	LOD (ppm)[1]	LOQ (ppm)[2]
Streptomycin	0.002 (all matrices except orange dried pulp) 0.01 (orange dried pulp)	0.01 (all matrices except orange dried pulp) 0.05 (orange dried pulp)

[1] LOD = limit of detection, estimated.
[2] LOQ = limit of quantitation, defined as the lowest fortification level where acceptable precision and accuracy data were obtained.

VI. Conclusions

The LC/MS/MS method, Method Report No. 033041-1 (Analytical Method for Streptomycin Residues in Citrus), was adequately validated for determining residues of streptomycin in citrus. Method validation data submitted in support of the method are also reviewed in conjunction with the citrus crop field trial and processing studies (MRID 49785502). A confirmatory method is not required because the method monitors two ion transitions. The submitted ILV study is acceptable. Adequate recoveries were obtained for whole oranges fortified with streptomycin at 0.01 ppm (LOQ) and 0.5 ppm (50x LOQ/proposed tolerance).

B.5.2.1.2 Pre-Registration Method (Data-Gathering)

A complete method description and supporting method validation data (MRID 49785502) were submitted for analytical method for the assay of streptomycin in citrus. This method was used for analysis of streptomycin in the U.S. crop field trial and processing studies submitted under the current action (refer to 49785502.de1, B.7.6.1.1, and 49785502.de2, B.7.7.3.1).

B.5.2.2 Analytical Methods for Foodstuff of Animal Origin (Livestock Matrices; Annex IIA 4.3, Annex IIIA 5.3)

Not applicable.

B.5.2.3. Multiresidue Methods

Not applicable.

B.7.6 Residues Resulting from Supervised Trials (Annex IIA 6.3; Annex IIIA 8.3)

B.7.6.1 Residues in Target Crops

B.7.6.1.1. Citrus, Crop Group 10-10

Document ID:	MRID No. 49785502 PMRA No. Not available
Report:	Gibson, T. (2015) Magnitude of the Residues of Streptomycin in/on Citrus and Processed Fractions of Citrus Following Applications of FireWall™ 50 WP. Study Nos. PSM-14-03-02 and 033040. Unpublished study prepared by AgroSource, Inc. 349 p.
Guidelines:	EPA OCSPP Harmonized Test Guideline 860.1500 Crop Field Trials (August 1996) PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 9 – Crop Field Trials PMRA Regulatory Directive DIR2010-05 – Revisions to the Residue Chemistry Crop Field Trial Requirements OECD Guideline 509 Crop Field Trial (September 2009)
GLP Compliance:	No deviations from regulatory requirements were reported which would have an impact on the validity of the study.
Acceptability:	The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP# 431343.
Evaluator:	Peter Savoia HED/RAB V

Note: This Data Evaluation Record (DER) was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 6/22/16). The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

AgroSource, Inc. has submitted field trial data for streptomycin on lemon, grapefruit, and orange. Twenty-four field trials were conducted in the United States during the 2014 growing season. Five lemon field trials were conducted in the North American Free Trade Agreement (NAFTA) Growing Zones 3 (FL; 1 trial) and 10 (CA; 4 trials); six grapefruit trials were conducted in Zones 3 (FL; 3 trials), 6 (TX, 1 trial), and 10 (CA, 2 trials); and thirteen orange trials were conducted in Zones 3 (FL; 9 trials), 6 (TX, 1 trial), and 10 (CA, 3 trials).

Each trial consisted of one untreated and one treated plot. At each trial location, the treated plots received three foliar directed airblast applications of an 50% wettable powder (WP) formulation of streptomycin (FireWall™ 50 WP) at 0.447-0.462 lb ai/A/application for a total seasonal rate of 1.347-1.375 lb ai/A. Applications were made at retreatment intervals (RTIs) of 20-21 days using ground equipment in spray volumes of ~91-110 gal/A. No adjuvants were used. Samples

of lemon, grapefruit, and orange were harvested at maturity at a preharvest interval (PHI) of 60-62 days. At one trial each for grapefruit and orange, samples were harvested at additional PHIs of 40, 50, 70, and 80 days to assess residue decline.

All samples were maintained frozen at the testing facilities, during shipping to the laboratory, and were stored frozen until analysis. The field residue samples were stored frozen for a maximum of 184 days (6.1 months) for whole fruit, 107 days (3.5 months) for whole fruit without peel, and 113 days (3.7 months) for peel from harvest to extraction. Samples were analyzed the same day of extraction. Freezer storage stability data were generated concurrently with the orange field trial studies. The data indicate that residues of streptomycin were stable during frozen storage for up to 50 days (2.6 months) in/on whole fruit and 49 days (2.6 months) in/on whole fruit without peel and peel; no 0-day data were provided. In addition, acceptable concurrent storage stability data are available (refer to DP# 427630, 11/29/17, I. Negrón-Encarnación) indicating that residues of streptomycin are stable under frozen conditions for up to 327 days in/on grapefruit (RAC). These data are acceptable to support the storage conditions and durations of samples from the submitted field trials; however, storage stability studies should always include a 0-day sampling interval to establish the residue levels present at the time samples are placed into storage [see OPPTS 860.1380(d)(6)(i)].

Samples were analyzed for residues of streptomycin by high performance liquid chromatography method with tandem mass spectrometry detection (LS/MS/MS), using a method based on USDA FSIS SOP No.: CLG-AMG1.02, with minor modifications. Acceptable method validation and concurrent recoveries were reported for samples of whole fruit, whole fruit without peel, and peel fortified with streptomycin at 0.01-0.10 ppm, thus validating the method. The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) was 0.01 ppm for all citrus fruits. The fortification levels used in concurrent method recovery were adequate to bracket expected residue levels (within an order of magnitude for orange, grapefruit, and citrus peel). Concurrent recoveries and residues in treated samples were not corrected for residues in controls.

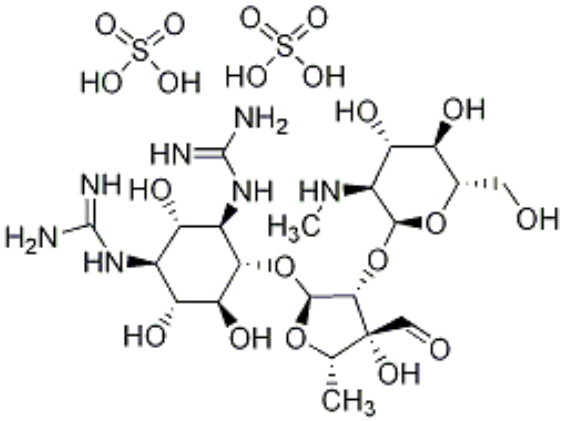
In citrus harvested at a 60- to 62- day PHI following three foliar directed applications of the 50% WP formulation of streptomycin at a total seasonal rate of 1.347-1.375 lb ai/A, residues (and per-trial averages) of streptomycin were <0.01-0.089 (<0.01-0.069) ppm in/on lemon, <0.01-0.478 (<0.01-0.414) ppm in/on grapefruit, <0.01-0.152 (<0.01-0.144) ppm in/on orange, <0.01-0.478 (<0.01-0.414) ppm in/on whole citrus fruit, <0.01-0.017 (<0.01-0.016) ppm in/on whole fruit without peel, and <0.01-1.12 (<0.015-1.08) ppm in/on peel.

In the residue decline trials, residues of streptomycin decreased to LOQ (<0.01 ppm) in/on grapefruit and decreased with increasing PHI in/on orange.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.1.1-1. Nomenclature for Streptomycin	
Common name	Streptomycin sulfate, streptomycin
Identity	O-2-deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-O-5-deoxy-3-C-formyl-α-L-lyxofuranosyl-(1→4)-N,N'-bis(aminoiminomethyl)-D-streptamine
CAS nos.	3810-74-0
Company experimental name	Not applicable
Other synonyms (if applicable)	Not applicable



B. Study Design

1. Test Procedure

Twenty-four citrus field trials were conducted with a 50% WP formulation of streptomycin during the 2014 growing season. Field trial locations by NAFTA growing zone are summarized in Table B.7.6.1.1-2. All trials, except for those listed in the table below, were separated by >20 miles and are therefore, considered independent (568_Criteria for Independence of Trials 04/23/2013 (EPA and PMRA)). The trials separated by <20 miles have been assessed for independence as detailed in the table below. It is noted that an addendum to the field trail study was submitted indicating that a separate tank mix was prepared for each field trial.

Independent Trial Determination[1]			
Crop	Trial Nos.	Differences	Decision
Orange	FL01 FL02 FL03 FL04 FL05 FL06 FL07 FL08 FL09	Same location Variety 01 (Tangerine) 02 (Tanglo) 03 (Kumquat) 04 (Ambersweet) 05 (Temple) 06 (Pumelo) 07 (Venecia) 08 (Navel) 09 (Hamlin) *No other differences in criteria	Separate due to variety; these are all various citrus cultivars (cultivated varieties) of oranges, lemons, and grapefruit. They all differ in size, fruit color, sweetness, and/or seasonal maturation (early vs late).
	CA16 & CA17	Location <20 miles Variety: Becks vs. Autumn Gold *No other differences in criteria	Separate due to variety; becks is an early developing navel orange whereas autumn gold is a late developing navel orange.
Lemon	CA20, CA21, & CA22	Location <20 miles and same variety *No other differences in criteria	Separate due to grower constraints; there are only two commercial varieties of lemon grown in CA, Lisbon and Eureka. Most of production occurs in the southern part of the state where temperatures are warmer. CA grows 19000 acres of lemon, only 3000 acres in Tulare where the 3 trials in question were held. Lemon production is just the Lisbon variety in Tulare. HED would consider the trials independent given grower and variety constraints.
Grapefruit	FL11 & FL13	Same location Variety: Texas Star vs. Flame *No other differences in criteria	Separate due to variety; different Texas cultivars of red grapefruit in size and fruit color redness.
	CA23 & CA24	Location <20 miles Variety: Melogold vs. Star Ruby *No other differences in criteria	Separate due to variety; Melogold is a grapefruit with yellow fruit whereas Star Ruby is a grapefruit with red fruit.

[1] All assessments are based on the replicate trial guidance presented in draft memo 568_Criteria for Independence of Trials 04/23/2013 (EPA and PMRA).

Table B.7.6.1.1-2. Trial Numbers and Geographical Locations.

Crop	No. Trials	NAFTA Growing Zone												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
Lemon	Sub.			1							4			5
	Req.			1							4			5
Orange	Sub.			9			1				3			13
	Req.[1]			11/8			1/1				4/3			16/12
Grapefruit	Sub.			3			1				2			6
	Req.[1]			5/3			1/1				2/2			8/6

[1] As per Table 5 of 860.1500, the second number reflects a 25% reduction in the number of field trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance as applicable (refer to Tables 2 and 3) or when application results in no quantifiable residues.

Locations and detailed use patterns for the trials are provided in Table B.7.6.1.1-3.

Table B.7.6.1.1-3. Study Use Pattern.							
Location: City, State; Year (Trial ID)	End-use Product[1]	Method of Application/ Timing of Application	Volume (gal/A)	Rate per Application (lb ai/A)	Retreat-ment Interval (days)	Total Rate (lb ai/A)	Surfactant/ Adjuvant
Lemon							
DeLeon Springs, FL; 2014 (10)	50% WP	1. Foliar directed airblast; BBCH 70	106	0.450	--	1.351	None
		2. Foliar directed airblast; BBCH 74	100	0.450	21		None
		3. Foliar directed airblast; BBCH 79	92	0.451	21		None
Reedley, CA; 2014 (19)	50% WP	1. Foliar directed airblast; BBCH 74	101	0.453	--	1.363	None
		2. Foliar directed airblast; BBCH 81	101	0.454	21		None
		3. Foliar directed airblast; BBCH 81	101	0.456	21		None
Woodlake, CA; 2014 (20)	50% WP	1. Foliar directed airblast; BBCH 79	100	0.451	--	1.351	None
		2. Foliar directed airblast; BBCH 81	100	0.450	21		None
		3. Foliar directed airblast; BBCH 81	100	0.450	21		None
Lindsay, CA; 2014 (21)	50% WP	1. Foliar directed airblast; BBCH 79	101	0.455	--	1.361	None
		2. Foliar directed airblast; BBCH 81	101	0.457	21		None
		3. Foliar directed airblast; BBCH 81	100	0.449	21		None
Porterville, CA; 2014 (22)	50% WP	1. Foliar directed airblast; BBCH 79	100	0.452	--	1.356	None
		2. Foliar directed airblast; BBCH 81	100	0.450	21		None
		3. Foliar directed airblast; BBCH 81	101	0.454	21		None
Grapefruit							
DeLeon Springs, FL; 2014 (11)	50% WP	1. Foliar directed airblast; BBCH 72	106	0.451	--	1.353	None
		2. Foliar directed airblast; BBCH 75	93	0.451	21		None
		3. Foliar directed airblast; BBCH 79	92	0.451	21		None
Clermont, FL; 2014 (12)	50% WP	1. Foliar directed airblast; BBCH 70	106	0.451	--	1.353	None
		2. Foliar directed airblast; BBCH 74	93	0.451	21		None
		3. Foliar directed airblast; BBCH 77	91	0.451	21		None
DeLeon Springs, FL; 2014 (13)	50% WP	1. Foliar directed airblast; BBCH 72	106	0.450	--	1.353	None
		2. Foliar directed airblast; BBCH 74	93	0.452	21		None
		3. Foliar directed airblast; BBCH 79	91	0.451	21		None
Alamo, TX; 2014 (15)	50% WP	1. Foliar directed airblast; BBCH 77	107	0.462	--	1.375	None
		2. Foliar directed airblast; BBCH 79	107	0.456	21		None
		3. Foliar directed airblast; BBCH 85	109	0.457	20		None
Exeter, CA; 2014 (23)	50% WP	1. Foliar directed airblast; BBCH 79	101	0.453	--	1.363	None
		2. Foliar directed airblast; BBCH 81	101	0.456	21		None
		3. Foliar directed airblast; BBCH 83	101	0.454	21		None
Lindsay, CA; 2014 (24)	50% WP	1. Foliar directed airblast; BBCH 79	100	0.452	--	1.347	None
		2. Foliar directed airblast; BBCH 81	99	0.448	21		None
		3. Foliar directed airblast; BBCH 81	99	0.447	21		None
Orange							
DeLeon Springs, FL; 2014 (01)	50% WP	1. Foliar directed airblast; BBCH 75	106	0.451	--	1.353	None
		2. Foliar directed airblast; BBCH 76	93	0.451	21		None
		3. Foliar directed airblast; BBCH 78	91	0.451	21		None
DeLeon Springs, FL; 2014 (02)	50% WP	1. Foliar directed airblast; BBCH 72	106	0.450	--	1.352	None
		2. Foliar directed airblast; BBCH 76	93	0.451	21		None
		3. Foliar directed airblast; BBCH 78	92	0.451	21		None

Table B.7.6.1.1-3. Study Use Pattern.

Location: City, State; Year (Trial ID)	End-use Product[1]	Method of Application/ Timing of Application	Volume (gal/A)	Rate per Application (lb ai/A)	Retreat-ment Interval (days)	Total Rate (lb ai/A)	Surfactant/ Adjuvant
DeLeon Springs, FL; 2014 (03)	50% WP	1. Foliar directed airblast; BBCH 72	106	0.450	--	1.352	None
		2. Foliar directed airblast; BBCH 76	93	0.451	21		None
		3. Foliar directed airblast; BBCH 79	92	0.451	21		None
DeLeon Springs, FL; 2014 (04)	50% WP	1. Foliar directed airblast; BBCH 70	106	0.451	--	1.353	None
		2. Foliar directed airblast; BBCH 76	93	0.451	21		None
		3. Foliar directed airblast; BBCH 79	91	0.451	21		None
DeLeon Springs, FL; 2014 (05)	50% WP	1. Foliar directed airblast; BBCH 72	106	0.451	--	1.352	None
		2. Foliar directed airblast; BBCH 76	93	0.450	21		None
		3. Foliar directed airblast; BBCH 79	92	0.451	21		None
DeLeon Springs, FL; 2014 (06)	50% WP	1. Foliar directed airblast; BBCH 72	106	0.451	--	1.354	None
		2. Foliar directed airblast; BBCH 76	93	0.452	21		None
		3. Foliar directed airblast; BBCH 79	92	0.451	21		None
DeLeon Springs, FL; 2014 (07)	50% WP	1. Foliar directed airblast; BBCH 70	106	0.450	--	1.353	None
		2. Foliar directed airblast; BBCH 73	93	0.452	21		None
		3. Foliar directed airblast; BBCH 79	92	0.451	21		None
DeLeon Springs, FL; 2014 (08)	50% WP	1. Foliar directed airblast; BBCH 70	106	0.451	--	1.353	None
		2. Foliar directed airblast; BBCH 70	93	0.451	21		None
		3. Foliar directed airblast; BBCH 79	92	0.451	21		None
DeLeon Springs, FL; 2014 (09)	50% WP	1. Foliar directed airblast; BBCH 70	106	0.452	--	1.354	None
		2. Foliar directed airblast; BBCH 70	93	0.452	21		None
		3. Foliar directed airblast; BBCH 79	91	0.450	21		None
Alamo, TX; 2014 (14)	50% WP	1. Foliar directed airblast; BBCH 77	105	0.456	--	1.370	None
		2. Foliar directed airblast; BBCH 79	106	0.453	21		None
		3. Foliar directed airblast; BBCH 85	110	0.461	20		None
Sanger, CA; 2014 (16)	50% WP	1. Foliar directed airblast; BBCH 74	101	0.454	--	1.357	None
		2. Foliar directed airblast; BBCH 81	100	0.453	21		None
		3. Foliar directed airblast; BBCH 81	100	0.450	21		None
Orosi, CA; 2014 (17)	50% WP	1. Foliar directed airblast; BBCH 79	102	0.461	--	1.372	None
		2. Foliar directed airblast; BBCH 81	102	0.459	21		None
		3. Foliar directed airblast; BBCH 81	100	0.452	21		None
Porterville, CA; 2014 (18)	50% WP	1. Foliar directed airblast; BBCH 79	100	0.451	--	1.367	None
		2. Foliar directed airblast; BBCH 81	103	0.462	21		None
		3. Foliar directed airblast; BBCH 81	101	0.454	21		None

[1] A 50% wettable powder (WP) formulation of streptomycin (FireWall™ 50 WP) was used. The product contains 65.8% of the active ingredient, streptomycin sulfate, which is equivalent to 50% streptomycin. Application rates, as reported by the petitioner, are as streptomycin sulfate. The target rate is 0.452 lb streptomycin sulfate/A/application which is equivalent to 0.344 lb streptomycin/A/application.

Citrus fruits were grown and maintained according to typical agricultural practices. Irrigation was used to supplement rainfall as needed. No unusual weather conditions were reported to have adversely affected crop production or yield during the study, and no phytotoxic responses were attributed to the use of streptomycin.

Sample Handling and Preparation

Single control and duplicate treated samples of lemon, grapefruit, and orange were harvested at PHIs of 60-62 days. At one trial each for grapefruit and orange, samples were harvested at additional PHIs of 40, 50, 70, and 80 days to assess residue decline. Samples were placed in frozen storage at the field sites within ~1 hour of harvest and were shipped frozen within 2-36 days of collection via ACDS freezer truck to the analytical laboratory, Ricerca Biosciences LLC (Concord, OH). At the analytical laboratory samples were homogenized in the presence of dry ice and were stored frozen (<-10 °C) until extraction for analysis.

2. Description of Analytical Procedures

Samples were analyzed for residues of streptomycin by LC/MS/MS, using a method based on "Analytical Method for Streptomycin Residues in Grapefruit" which was based on USDA FSIS SOP No.: CLG-AMG1.02. Minor modifications included the use of pectinase and cellulase added to the samples to aid in extraction. A complete description of the method was included in the submission.

Briefly, samples were extracted with phosphate buffer (pH 4), pectinase and cellulase were added, and then the extract was filtered. The filtrate was purified on a C8 solid phase extraction (SPE) column. The resulting eluate was adjusted to pH ~8 and applied to a CBX SPE column for further purification. Residues were eluted with 1% formic acid in methanol. The eluate was evaporated to near dryness and reconstituted in 0.1% formic acid in water for LC/MS/MS analysis. The method monitors the following ion transition for determination of streptomycin: *m/z* 582.3 → 263.1.

The LOQ (determined as the LLMV) was 0.01 ppm, and the limit of detection (LOD) was 0.002 ppm.

II. RESULTS AND DISCUSSION

Method performance was evaluated during method validation and by use of concurrent recovery samples. Samples of untreated whole fruit, whole fruit without peel, and peel were fortified with streptomycin at 0.01 and 0.1 ppm for method validation and for concurrent recoveries. Recoveries were generally within the acceptable range of 70-120%. The method was considered valid for the analysis of streptomycin residues in/on citrus fruit matrices (Table B.7.6.1.1-4). The fortification levels bracketed the measured residues (within an order of magnitude for orange, grapefruit, and citrus peel). Concurrent recoveries were not corrected for apparent residues in controls.

The detector response was linear (coefficient of determination was $r^2 \geq 0.9946$ within the calibration range of 1-100 ng/mL). Representative chromatograms of control samples, fortified samples, and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Apparent

residues in controls were below the LOQ (<0.01 ppm) for all citrus fruits. The reported residue values were not corrected for apparent residues in controls.

Table B.7.6.1.1-4. Summary of Method Validation and Procedural/Concurrent Recoveries of Streptomycin from Citrus Fruits.

Matrix	Analyte	Fortification Level[1] (ppm)	Sample size (n)	Recoveries[1] (%)	Mean ± Std. Dev. (%)
Method validation					
Citrus, whole fruit	Streptomycin	0.01-0.10	6	64; 76-108	89 ± 16.8
Citrus, whole fruit without peel		0.01-0.10	6	86-114	106 ± 10.7
Citrus, peel		0.01-0.10	6	70-89	77 ± 7.0
Concurrent recoveries					
Citrus, whole fruit	Streptomycin	0.01-0.10	18	69.5; 73.6-119.5; 123.6	96.9 ± 15.7
Citrus, whole fruit without peel		0.01-0.10	10	72.3-103.7	89.3 ± 9.6
Citrus, peel		0.01-0.10	10	69.6; 70.8-105.1	81.1 ± 12.6

[1] Concurrent recoveries were not corrected for apparent residues in controls.

The field residue samples were stored frozen for a maximum of 184 days (6.1 months) for whole fruit, 107 days (3.5 months) for whole fruit without peel, and 113 days (3.7 months) for peel from harvest to extraction (Table B.7.6.1.1-5a). Samples were analyzed the same day of extraction. Freezer storage stability data were generated concurrently with the orange field trial studies. The data indicate (Table B.7.6.1.1-5b) that residues of streptomycin were stable during frozen storage for up to 50 days (2.6 months) in/on whole fruit and 49 days (2.6 months) in/on whole fruit without peel and peel; no 0-day data were provided. In addition, acceptable concurrent storage stability data are available (refer to DP# 427630, 11/29/17, I. Negrón-Encarnación) indicating that residues of streptomycin are stable under frozen conditions for up to 327 days in/on grapefruit (RAC). These data are acceptable to support the storage conditions and durations of samples from the submitted field trials; however, storage stability studies should always include a 0-day sampling interval to establish the residue levels present at the time samples are placed into storage [see OPPTS 860.1380(d)(6)(i)].

Table B.7.6.1.1-5a. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability
Citrus, whole fruit	<-10	75-184 days (2.5-6.1 months)	Residues of streptomycin are stable for up to 50 days in/on whole fruit and 49 days (1.6 months) in/on whole fruit without peel and peel.[2] In addition, residues of streptomycin are stable in/on grapefruit (RAC) under frozen conditions for up to 327 days.[3]
Citrus, whole fruit without peel		93-107 days (3.1-3.5 months)	
Citrus, peel		100-113 days (3.3-3.7 months)	

[1] Interval from harvest to extraction. Samples were analyzed the same day of extraction.
[2] Based on concurrent storage stability study. See Table B.7.6.1.1-5b.
[3] Refer to DP#427630, 11/29/17, I. Negrón-Encarnación.

Table B.7.6.1.1-5b. Stability of Streptomycin Residues in Orange Stored Frozen (≤-10 °C).						
Commodity	Spike Level (ppm)	Storage interval (days)	Fresh Fortification Recovery (%)	Stored Sample Recoveries (%)	Mean Recovery (%)	Corrected % recovery[1]
Orange, whole fruit	0.01	50	72.4	70.1	94.1	118.2
	0.10		86.7	118.0		
Orange, whole fruit without peel	0.01	49	98.1	99.2	104.2	91.4
	0.10		129.9	109.1		
Orange, peel	0.01	49	89.4	98.2	107.6	105.0
	0.10		115.4	116.9		

[1] Corrected for recovery in freshly fortified samples.

The results from the submitted field trials are presented in Tables B.7.6.1.1-6 and B.7.6.1.1-7. In citrus harvested at a 60- to 62- day PHI following three foliar directed applications of the 50% WP formulation of streptomycin at a total seasonal rate of 1.347-1.375 lb ai/A, residues (and per-trial averages) of streptomycin were <0.01-0.089 (<0.01-0.069) ppm in/on lemon, <0.01-0.478 (<0.01-0.414) ppm in/on grapefruit, <0.01-0.152 (<0.01-0.144) ppm in/on orange, <0.01-0.478 (<0.01-0.414) ppm in/on whole citrus fruit, <0.01-0.017 (<0.01-0.016) ppm in/on whole fruit without peel, and <0.01-1.12 (<0.015-1.08) ppm in/on peel.

In the residue decline trials, residues of streptomycin decreased to LOQ (<0.01 ppm) in/on grapefruit and decreased with increasing PHI in/on orange.

Table B.7.6.1.1-6. Residue Data from Citrus Fruit Trials with Streptomycin.[1]						
Location: City, State; Year (Trial ID)	Zone	Crop Variety	Matrix	Total Rate (lb ai/A)	PHI (days)	Residues[2] ppm [Average]
Lemon						
DeLeon Springs, FL; 2014 (10)	3	Meyer	Whole fruit	1.351	62	0.079, 0.030 [0.055]
			Whole fruit without peel			(0.0089), (0.0066) [<0.01]
			Peel			0.292, 0.111 [0.202]
Reedley, CA; 2014 (19)	10	Lisbon	Whole fruit	1.363	60	0.048[3], 0.089 [0.069]
			Whole fruit without peel			ND, (0.0032) [<0.01]
			Peel			0.020[3], 0.069 [0.045]
Woodlake, CA; 2014 (20)	10	Lisbon	Whole fruit	1.351	60	0.030[3], 0.012[3] [0.021]
			Whole fruit without peel			ND, ND [<0.01]
			Peel			0.057[3], 0.026[3] [0.042]
Lindsay, CA; 2014 (21)	10	Lisbon	Whole fruit	1.361	60	(0.0026)[3], ND, [<0.01]
			Whole fruit without peel			ND, (0.0037) [<0.01]
			Peel			0.022[3], 0.044[3] [0.033]
Porterville, CA; 2014 (22)	10	Lisbon	Whole fruit	1.356	60	ND, 0.010[3] [<0.01]
			Whole fruit without peel			ND, ND [<0.01]
			Peel			ND, (0.0078)[3] [<0.01]
Grapefruit						
DeLeon Springs, FL; 2014 (11)	3	Texas Star	Whole fruit	1.353	62	0.030, 0.040 [0.035]
			Whole fruit without peel			ND, (0.0025) [<0.01]
			Peel			0.058[3], 0.050[3] [0.054]

Table B.7.6.1.1-6. Residue Data from Citrus Fruit Trials with Streptomycin.[1]						
Location: City, State; Year (Trial ID)	Zone	Crop Variety	Matrix	Total Rate (lb ai/A)	PHI (days)	Residues[2] ppm [Average]
Clermont, FL; 2014 (12)	3	Texas Star	Whole fruit	1.353	62	0.028, 0.011 [0.020]
			Whole fruit without peel			(0.0066), (0.0023) [<0.01]
			Peel			0.037[3], 0.023[3] [0.030]
DeLeon Springs, FL; 2014 (13)	3	Flame	Fruit	1.353	40	0.063[3], 0.022[3] [0.043]
					50	0.052[3], (0.0029)[3] [<0.031]
					62	0.022[3], 0.041[3] [0.032]
					70	0.021[3], ND [<0.016]
					80	ND, ND [<0.01]
Alamo, TX; 2014 (15)	6	Ruby Red	Whole fruit	1.375	61	0.349, 0.478 [0.414]
			Whole fruit without peel			(0.0089), 0.013 [<0.012]
			Peel			1.037, 1.122 [1.080]
Exeter, CA; 2014 (23)	10	Melogold	Whole fruit	1.363	60	0.029[3], 0.025[3] [0.027]
			Whole fruit without peel			ND, ND [<0.01]
			Peel			0.036[3], 0.018[3] [0.027]
Lindsay, CA; 2014 (24)	10	Star Ruby	Whole fruit	1.347	60	ND, ND [<0.01]
			Whole fruit without peel			ND, ND [<0.01]
			Peel			(0.0069)[3], 0.020[3] [<0.015]
Orange						
DeLeon Springs, FL; 2014 (01)	3	Tangerine	Whole fruit	1.353	62	0.073, 0.104 [0.089]
			Whole fruit without peel			0.011, 0.011 [0.011]
			Peel			0.240, 0.241 [0.241]
DeLeon Springs, FL; 2014 (02)	3	Tanglo	Whole fruit	1.352	62	0.136, 0.152 [0.144]
			Whole fruit without peel			0.016, 0.016 [0.016]
			Peel			1.057, 0.572 [0.815]
DeLeon Springs, FL; 2014 (03)	3	Kumquat	Whole fruit	1.352	62	(0.0027)[3], ND [<0.01]
			Whole fruit without peel			(0.0091), ND [<0.01]
			Peel			0.465, 0.192 [0.329]
DeLeon Springs, FL; 2014 (04)	3	Ambersweet	Whole fruit	1.353	62	0.089, 0.063[3] [0.076]
			Whole fruit without peel			(0.0067), (0.0068) [<0.01]
			Peel			0.173, 0.247 [0.210]
DeLeon Springs, FL; 2014 (05)	3	Temple	Whole fruit	1.352	62	0.102, 0.020 [0.061]
			Whole fruit without peel			0.017[3], (0.0033)[3] [<0.014]
			Peel			0.481, 0.031[3] [0.256]
DeLeon Springs, FL; 2014 (06)	3	Pumelo	Whole fruit	1.354	62	0.018, 0.025 [0.022]
			Whole fruit without peel			(0.0024), ND [<0.01]
			Peel			0.036[3], 0.063[3] [0.050]
DeLeon Springs, FL; 2014 (07)	3	Venecia	Whole fruit	1.353	62	0.123, 0.113 [0.118]
			Whole fruit without peel			(0.0060)[3], (0.0051)[3] [<0.01]
			Peel			0.214, 0.126 [0.170]
DeLeon Springs, FL; 2014 (08)	3	Navel	Fruit	1.353	40	0.063[3], 0.100 [0.082]
					50	0.102, 0.044[3] [0.073]
					62	0.056[3], 0.043[3] [0.050]
					70	0.044[3], 0.043[3] [0.044]
					80	0.021[3], 0.023[3] [0.022]

Table B.7.6.1.1-6. Residue Data from Citrus Fruit Trials with Streptomycin.[1]						
Location: City, State; Year (Trial ID)	Zone	Crop Variety	Matrix	Total Rate (lb ai/A)	PHI (days)	Residues[2] ppm [Average]
DeLeon Springs, FL; 2014 (09)	3	Hamlin	Whole fruit	1.354	62	0.085, 0.051 [0.068]
			Whole fruit without peel			(0.0044)[3], (0.0037)[3] [<0.01]
			Peel			0.119, 0.053[3] [0.086]
Alamo, TX; 2014 (14)	6	Valencia	Whole fruit	1.370	61	0.031, 0.045 [0.038]
			Whole fruit without peel			(0.0032), (0.0026) [<0.01]
			Peel			0.148, 0.177 [0.163]
Sanger, CA; 2014 (16)	10	Becks	Whole fruit	1.357	60	0.133, 0.070[3] [0.102]
			Whole fruit without peel			(0.0040), ND [<0.01]
			Peel			0.084, 0.116 [0.100]
Orosi, CA; 2014 (17)	10	Autumn Gold	Whole fruit	1.372	60	ND, 0.030[3] [<0.020]
			Whole fruit without peel			ND, ND [<0.01]
			Peel			0.115, 0.075[3] [0.095]
Porterville, CA; 2014 (18)	10	Navel	Whole fruit	1.367	60	0.017[3], 0.046[3] [0.032]
			Whole fruit without peel			ND, ND [<0.01]
			Peel			0.023[3], 0.050[3] [0.037]

[1] A 50% wettable powder (WP) formulation of streptomycin (FireWall 50 WP) was used.
[2] ND = Nondetectable (<LOD). The LOQ was 0.01 ppm and the LOD was 0.002 ppm. Residues between the LOD and LOQ are reported in parentheses. Per-trial averages and combined residues were calculated by the study reviewer using the LOQ for residues <LOQ.
[3] Mean of duplicate analyses.

Table B.7.6.1.1-7. Summary of Residues from Citrus Fruit Trials with Streptomycin.										
Crop Matrix	Total Application Rate (lb ai/A)	PHI (days)	n[1]	Residues (ppm)						
				Min.[2]	Max.[2]	LAFT[3]	HAFT[3]	Median[3]	Mean[3]	SD[3]
Lemon	1.351-1.363	60-62	5	<0.01	0.089	<0.01	0.069	0.021	0.033	0.027
Grapefruit	1.347-1.375	60-62	6	<0.01	0.478	<0.01	0.414	0.029	0.089	0.159
Orange	1.352-1.372	60-62	13	<0.01	0.152	<0.01	0.144	0.061	0.064	0.041
Citrus, whole fruit	1.347-1.375	60-62	24	<0.01	0.478	<0.01	0.414	0.037	0.064	0.083
Citrus, whole fruit without peel			22	<0.01	0.017	<0.01	0.016	0.010	0.011	0.0015
Citrus, peel			22	<0.01	1.12	<0.01	1.08	0.091	0.186	0.266

[1] n = number of field trials.
[2] Values based on total number of samples.
[3] Values based on per-trial averages. LAFT = lowest average field trial, HAFT = highest average field trial, SD = standard deviation. For computation of the LAFT, HAFT, median, mean, and standard deviation, values < LOQ are assumed to be at the LOQ (0.01 ppm).

III. CONCLUSIONS

The citrus fruit field trials are considered scientifically acceptable. In citrus harvested at a 60- to 62- day PHI following three foliar directed applications of the 50% WP formulation of streptomycin at a total seasonal rate of 1.347-1.375 lb ai/A, residues (and per-trial averages) of streptomycin were <0.01-0.089 (<0.01-0.069) ppm in/on lemon, <0.01-0.478 (<0.01-0.414) ppm in/on grapefruit, <0.01-0.152 (<0.01-0.144) ppm in/on orange, <0.01-0.478 (<0.01-0.414) ppm in/on whole citrus fruit, <0.01-0.017 (<0.01-0.016) ppm in/on whole fruit without peel, and <0.01-1.12 (<0.015-1.08) ppm in/on peel.

In the residue decline trials, residues of streptomycin decreased to LOQ (<0.01 ppm) in/on grapefruit and decreased with increasing PHI in/on orange.

An acceptable method was used for residue quantitation, and adequate storage stability data are available to support sample storage durations and conditions.

REFERENCES

DP#427630, 11/29/17, I. Negrón-Encarnación

B.7.7 Effects of Industrial Processing and/or Household Preparation (Annex IIA 6.5; Annex IIIA 8.5)

B.7.7.1 Nature of the Residue (Annex IIA 6.5.1; Annex IIIA 8.5.1) – *Not a data requirement in North America*

B.7.7.2 Distribution of the Residue in Peel/Pulp (Annex IIA 6.5.2; Annex IIIA 8.5.2)

To be included if available.

B.7.7.3 Magnitude of Residues on Set of Representative Processes (Annex IIA 6.5.3; Annex IIIA 8.5.3)

B.7.7.3.1 Orange

Document ID:	MRID No. 49785502 PMRA No. Not Applicable
Report:	Gibson, T. (2015) Magnitude of the Residues of Streptomycin in/on Citrus and Processed Fractions of Citrus Following Applications of FireWall™ 50 WP. Study Nos. PSM-14-03-02 and 033040. Unpublished study prepared by AgroSource, Inc. 349 p.
Guidelines:	EPA OCSPP Harmonized Test Guideline 860.1520 Processed Food/Feed (August 1996) PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 10 – Processed Food/Feed OECD Guideline 508 Magnitude of the Pesticide Residues in Processed Commodities (October 2008)
GLP Compliance:	No deviations from regulatory requirements were reported which would have an impact on the validity of the study.
Acceptability:	The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP# 431343.
Evaluator:	Peter Savoia HED/RAB V

Note: This Data Evaluation Record (DER) was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 6/22/16. The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

AgroSource, Inc. has submitted a processing study for streptomycin on orange from one trial conducted in FL during the 2014 growing season reflecting three foliar directed airblast applications of a 50% wettable powder (WP) formulation of streptomycin (FireWall™ 50 WP) made at an exaggerated rate of 1.35-1.36 lb ai/A/application for a total seasonal rate of 4.07 lb ai/A (corresponding to 3x the rate used in the corresponding field trials). Applications were made at a 21-day retreatment interval (RTI), in spray volumes of ~91-106 gal/A. No adjuvants

were used. Samples of mature orange were harvested at a preharvest interval (PHI) of 62 days and were processed into juice, dried pulp, and oil using simulated commercial practices by the University of Idaho Food Technology Center (Caldwell, ID). Adequate descriptions of the processing procedures were provided, including material balance summaries.

Samples of orange (RAC) were shipped fresh on the day of harvest to the processing facility, where they were stored cool until processing. Samples were stored frozen following processing, during shipping to the laboratory, and were stored frozen until analysis. Processing was completed within 3-6 days of harvest. The maximum storage intervals for samples between harvest/processing and extraction were 213 days for orange (RAC), 131 days for juice, 213 days for dried pulp, and 147 days for oil. Samples were analyzed within 0-1 days of extraction. Freezer storage stability data were generated concurrently with the orange field trial and processing studies. The data indicate that residues of streptomycin are stable for up to 50 days in/on whole fruit and 49 days (1.6 months) in/on whole fruit without peel and peel; no 0-day data were provided. In addition, residues of streptomycin are stable under frozen conditions for up to 327 days in/on grapefruit (RAC), juice for up to 481 days, and dried pulp for up to 720 days (refer to DP#427630, 11/29/17, I. Negrón-Encarnación). Residues were not stable in oil: recoveries were 49% at 474 days and 36% at 502 days. The available storage stability data are acceptable to support the sample storage conditions and durations of the samples from the submitted field trials.

Samples were analyzed for residues of streptomycin by high performance liquid chromatography with tandem mass spectrometry detection (LC/MS/MS), using a method based on "Analytical Method for Streptomycin Residues in Grapefruit" which was based on USDA FSIS SOP No: CLG-AMG1.02, with minor modifications. Acceptable method validation and concurrent recoveries were reported for samples fortified with streptomycin at 0.01-0.1 ppm for citrus (RAC), juice and peel, 0.05-1 ppm for dried pulp, and 0.01-1 ppm for oil, thus validating the method. The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) was 0.01 ppm for all orange matrices, except orange dried pulp. The LOQ for orange dried pulp is 0.05 ppm. The fortification levels used in concurrent method recovery were adequate to bracket expected residue levels (within an order of magnitude for oil). Concurrent recoveries and residues in treated samples were not corrected for residues in controls.

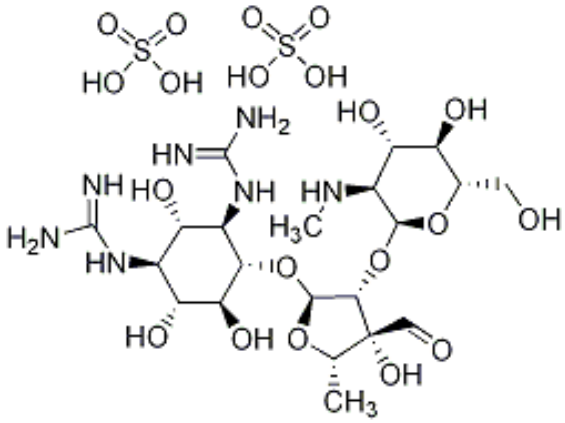
In one trial conducted in FL following three foliar directed applications of the 50% WP formulation of streptomycin at a total rate of 4.07 lb ai/A, average residues in/on whole fruit (RAC) were 0.098 ppm. In the processed commodities, average residues were below the LOQ (<0.01 ppm) in juice and oil, 0.681 ppm in dried pulp, and 0.236 ppm in peel. A comparison of the residues in whole fruit (RAC) with those in the processed orange fraction (juice, dried pulp, oil, and peel) indicated that residues of streptomycin concentrate in dried pulp (processing factor of 6.9x) and peel (2.4x) but do not concentrate in juice or oil (<0.1x).

The processing factors did not exceed the theoretical concentration factors for citrus of 3.3x for peel, 1000x for oil, and 2x for juice (based on separation into components, Table 3 of OCSPP 860.1520).

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.7.3.1-1. Nomenclature for Streptomycin	
Common name	Streptomycin sulfate, streptomycin
Identity	O-2-deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-O-5-deoxy-3-C-formyl-α-L-lyxofuranosyl-(1→4)-N,N'-bis(aminoiminomethyl)-D-streptamine
CAS nos.	3810-74-0
Company experimental name	Not applicable
Other synonyms (if applicable)	Not applicable



B. Study Design

1. Test Procedure

Location and detailed use pattern for the trial is provided in Table B.7.7.3.1-2.

Table B.7.7.3.1-2. Study Use Pattern.

Location: City, State; Year (Trial ID)	End-use Product[1]	Method of Application/ Timing of Application	Volume (gal/A)	Rate per Application[2] (lb ai/A)	Retreatment Interval (days)	Total Rate[2] (lb ai/A)	Surfactant/ Adjuvant
DeLeon Springs, FL; 2014 (09)	50% WP	1. Foliar directed airblast; BBCH 73	106	1.36	--	4.07	None
		2. Foliar directed airblast; BBCH 70	93	1.36	21		None
		3. Foliar directed airblast; BBCH 79	91	1.35	21		None

[1] A 50% wettable powder (WP) formulation of streptomycin (FireWall™ 50 WP) was used. The product contains 65.8% of the active ingredient streptomycin sulfate, which is equivalent to 50% streptomycin.
[2] Application rates were reported by the petitioner as streptomycin sulfate. The target rate is 1.356 lb streptomycin sulfate/A/application which is equivalent to 1.032 lb streptomycin/A/application.

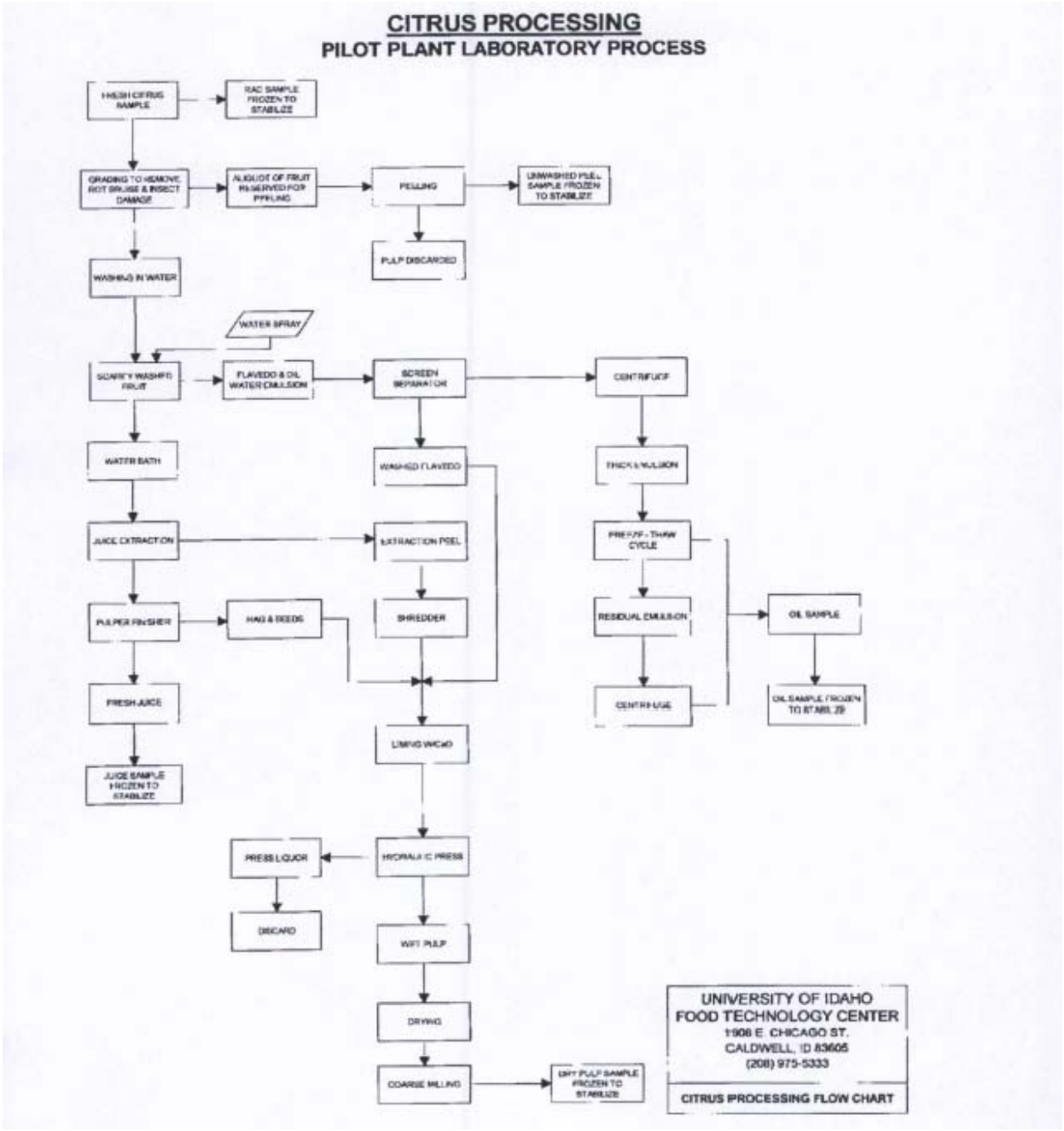
Sample Handling and Preparation

Single control and treated bulk samples (~100-175 kg) of orange (RAC) were harvested at a 62-day PHI and were shipped fresh overnight via FedEx to the processing facility, the University of Idaho Food Technology Center (Caldwell, ID), where they were stored cool (4 °C) until processing. Processing was completed within 3-6 days of harvest, after which samples were placed in frozen storage (-21 to -16 °C). The processed commodity samples were shipped frozen via ACDS freezer truck within 2-5 days of processing to the analytical laboratory, Ricerca Biosciences LLC (Concord, OH). At the analytical laboratory samples of orange and dried pulp were homogenized in the presence of dry ice and were stored frozen (<- 10 °C) until extraction for analysis.

Sample Processing

The processing procedures simulated commercial operations of orange production as closely as possible to generate the required fractions of orange (RAC) and the processed fractions of juice, dried pulp, and oil, with some variations to commercial methods. Adequate descriptions of processing procedures were provided, including material balance summaries. A processing flowchart for orange processed fractions, copied without alteration from MRID 49785502, is presented in Figure B.1.

FIGURE B.1. Processing Flowchart for Orange Juice, Dried Pulp, and Oil.



2. Description of Analytical Procedures

Samples were analyzed for residues of streptomycin by LC/MS/MS, using a method based on "Analytical Method for Streptomycin Residues in Grapefruit" which was based on USDA FSIS SOP No.: CLG-AMG1.02. Minor modifications included the use of pectinase and cellulase added to the samples to aid in extraction. A complete description of the method was included in the submission.

Briefly, samples orange, juice, and dried pulp were extracted with phosphate buffer (pH 4 for fruit and juice; pH 2 for dried pulp), pectinase and cellulase were added, and then the extract was filtered. The filtrate was purified on a C8 solid phase extraction (SPE) column. The resulting eluate was adjusted to pH ~8 and applied to a CBX SPE column for further purification. Residues were eluted with 1% formic acid in methanol. The eluate was evaporated to near dryness and reconstituted in 0.1% formic acid in water for LC/MS/MS analysis. For citrus oil, 0.1% formic acid in water was added to the sample and centrifuged. For 10x LOQ and 100x LOQ oil samples, a transfer pipette was pushed through the organic layer and an aliquot of the aqueous layer was collected and diluted with 0.1% formic acid for LC/MS/MS analysis. For LOQ oil samples, the supernatant was evaporated to dryness and diluted with 0.1% formic acid for LC/MS/MS analysis. The method monitors the following ion transition for determination of streptomycin: *m/z* 582.3 → 263.1.

The LOQ (determined as the LLMV) was 0.01 ppm, and the limit of detection (LOD) was 0.002 ppm for all matrices, except for orange dried pulp. For orange dried pulp, the LOQ was 0.05 ppm, and the LOD was 0.01 ppm.

II. RESULTS AND DISCUSSION

Method performance was evaluated during method validation and by use of concurrent recovery samples. For method validation and concurrent recovery, untreated samples were fortified with streptomycin at 0.01 and 0.1 ppm for orange (RAC), juice, and peel, 0.05, 0.5, and 1 ppm for dried pulp, and 0.01, 0.10, and 1 ppm for oil. Recoveries were generally within the acceptable range of 70-120%. The method was considered valid for the analysis of streptomycin residues in orange matrices (Table B.7.7.3.1-3). The fortification levels used in concurrent recovery bracketed the measured residues. Concurrent recoveries were not corrected for apparent residues in controls.

The detector response was linear for the RAC and processed commodities (coefficient of determination, $r^2 \geq 0.9946$ within the calibration range of 1-100 ng/mL). Representative chromatograms of control samples, fortified samples, and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Apparent residues in controls were below the LOQ in/on all matrices. The reported residue values were not corrected for apparent residues in controls.

Table B.7.7.3.1-3. Summary of Method Validation and Procedural/Concurrent Recoveries of Streptomycin from Orange Matrices.

Matrix	Analyte	Fortification Level (ppm)	Sample size (n)	Recoveries[1] (%)	Mean ± Std. Dev.[2] (%)
Method validation[3]					
Citrus, whole fruit	Streptomycin	0.01-0.1	6	64; 76-108	89 ± 16.8
Juice		0.01-0.1	6	91-115	102 ± 8.2
Dried pulp		0.05-0.5	6	62.6; 71.7-90.1	77.3 ± 10.4
Oil		0.01-1	9	78.5-111.4	94.8 ± 10.8
Peel		0.01-0.1	6	70-89	77 ± 7.0

Table B.7.7.3.1-3. Summary of Method Validation and Procedural/Concurrent Recoveries of Streptomycin from Orange Matrices.

Matrix	Analyte	Fortification Level (ppm)	Sample size (n)	Recoveries[1] (%)	Mean ± Std. Dev.[2] (%)
Concurrent recoveries					
Citrus, whole fruit	Streptomycin	0.01-0.1	2	109.0, 123.6	116.3
Juice		0.01-0.1	2	97.1, 80.0	88.6
Dried pulp		0.05-1	3	92.4-101.2	97.4 ± 4.5
Oil		0.01-1	3	83.1-108.7	92.4 ± 14.1
Peel		0.01-0.1	2	98.4, 105.1	101.8

[1] Concurrent recoveries were not corrected for apparent residues in controls.
[2] Standard deviation is not calculated for sample sizes <3.
[3] Method validation data were also reported in the residue analytical method DER [refer to 49785502, 03, 04, 05.DER in section B.5.2.1.1 Post-Registration Method (Enforcement)].

Processing was completed within 3-6 days of harvest. The maximum storage intervals for samples between harvest/processing and extraction were 213 days for orange (RAC), 131 days for juice, 213 days for dried pulp, and 147 days for oil (Table B.7.7.3.1-4a). Samples were analyzed within 0-1 days of extraction. Freezer storage stability data were generated concurrently with the orange field trial and processing studies. The data indicate (Table B.7.7.3.1-4b) that residues of streptomycin are stable for up to 50 days in/on whole fruit and 49 days (1.6 months) in/on whole fruit without peel and peel; no 0-day data were provided. In addition, acceptable concurrent storage stability data are available (refer to DP# 427630, 11/29/17, I. Negrón-Encarnación) indicating that residues of streptomycin are stable under frozen conditions for up to 327 days in/on grapefruit (RAC), juice for up to 481 days, and dried pulp for up to 720 days. Residues were not stable in oil: recoveries were 49% at 474 days and 36% at 502 days. The available storage stability data are acceptable to support the sample storage conditions and durations of the samples from the submitted field trials.

Table B.7.7.3.1-4a. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability
Orange (RAC)	-21 to -16 (processing facility) <-10 (laboratory)	213 days (7.0 months)	Residues of streptomycin are stable for up to 50 days in/on whole fruit and 49 days (2.6 months) in/on whole fruit without peel and peel.[2] In addition, acceptable concurrent storage stability data are available indicating that residues of streptomycin are stable under frozen conditions for up to 327 days in/on grapefruit (RAC), juice for up to 481 days, and dried pulp for up to 720 days. Residues were not stable in oil: recoveries were 49% at 474 days and 36% at 502 days.[3]
Juice		131 days (4.3 months)	
Dried pulp		213 days (7.0 months)	
Oil		147 days (4.8 months)	

[1] Interval from harvest to extraction or processing. Samples were analyzed 0-1 days of extraction.
[2] Based on concurrent storage stability study. See Table B.7.7.3.1-4b.
[3] Refer to DP#427630, 11/29/17, I. Negrón-Encarnación.

Table B.7.7.3.1-4b. Stability of Streptomycin Residues in Orange Stored Frozen (≤-10 °C).						
Commodity	Spike Level (ppm)	Storage Interval (days)	Fresh Fortification Recovery (%)	Stored Sample Recoveries (%)	Mean Recovery (%)	Corrected % recovery[1]
Orange, whole fruit	0.01	50	72.4	70.1	94.1	118.2
	0.10		86.7	118.0		
Orange, whole fruit without peel	0.01	49	98.1	99.2	104.2	91.4
	0.10		129.9	109.1		
Orange, peel	0.01	49	89.4	98.2	107.6	105.0
	0.1		115.4	116.9		

[1] Corrected for average recovery in freshly fortified samples.

Residues found in samples and processing factors are given in Table B.7.7.3.1-5. In one trial conducted in FL following three foliar directed applications of the 50% WP formulation of streptomycin at a total rate of 4.07 lb ai/A, average residues in/on whole fruit (RAC) were 0.098 ppm. In the processed commodities, average residues were below the LOQ (<0.01 ppm) in juice and oil, 0.681 ppm in dried pulp, and 0.236 ppm in peel. A comparison of the residues in whole fruit (RAC) with those in the processed orange fraction (juice, dried pulp, oil, and peel) indicated that residues of streptomycin concentrate in dried pulp (processing factor of 6.9x) and peel (2.4x) but do not concentrate in juice or oil (<0.1x).

Table B.7.7.3.1-5. Residue Data from Orange Processing Study with Streptomycin.			
Commodity	Residues[1] (ppm) [Average]	Processing Factor[2]	Median Processing Factor[3]
Orange (RAC)	0.101, 0.095 [0.098]	--	--
Juice	(0.00406), (0.00573) [<0.01]	<0.1x	Not applicable
Dried pulp	0.573, 0.788 [0.681]	6.9x	
Oil[4]	(0.00264), (0.00831) [<0.01]	<0.1x	
Peel	0.214[5], 0.258[6] [0.236]	2.4x	

[1] The LOQ was 0.01 ppm, and the LOD was 0.002 ppm for all matrices, except orange dried pulp. The LOQ was 0.05 ppm, and the LOD was 0.01 ppm for orange dried pulp. Residues between the LOD and LOQ are reported in parentheses. Per-trial averages were calculated by the study reviewer using the LOQ for values reported as <LOQ.
[2] Processing Factor = [Measured residue for analyte in the processed fraction] / [Measured residue for analyte in the RAC].
[3] Median processing factor for both plots and all sites.
[4] The available storage stability data indicate that residues of streptomycin were not stable in oil (recoveries were 49% at 474 days and 36% at 502 days); however, residues were not corrected for loss on storage because residues were below the LOQ.
[5] Mean of duplicate dilution analyses.
[6] Result of one dilution only.

III. CONCLUSIONS

The orange processing study is considered scientifically acceptable. In one trial conducted in FL following three foliar directed applications of the 50% WP formulation of streptomycin at a total rate of 4.07 lb ai/A, average residues in/on whole fruit (RAC) were 0.098 ppm. In the processed commodities, average residues were below the LOQ (<0.01 ppm) in juice and oil, 0.681 ppm in dried pulp, and 0.236 ppm in peel. A comparison of the residues in whole fruit (RAC) with those in the processed orange fraction (juice, dried pulp, oil, and peel) indicated that residues of streptomycin concentrate in dried pulp (processing factor of 6.9x) and peel (2.4x) but do not concentrate in juice or oil (<0.1x).

The processing factors did not exceed the theoretical concentration factors for citrus of 3.3x for peel, 1000x for oil, and 2x for juice (based on separation into components, Table 3 of OCSPP 860.1520).

An acceptable method was used for residue quantitation, and acceptable storage stability data are available to support sample storage durations and conditions.

REFERENCES

DP#427630, 11/29/17, I. Negrón-Encarnación